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Short-Chain Fatty Acids as Sanitizers for Beef[1,2,3]

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(Received for publication June 1, 1979)

ABSTRACT

Formic, acetic and propionic acids in various combinations and individually were screened for antimicrobial efficacy and effect on meat color. Microorganisms tested were two pseudomonads, three coliforms, a *Streptococcus* sp., a *Micrococcus* sp., a spore-forming bottom yeast and three film yeasts which reproduced by budding. Variables tested were microorganism, pH, concentration of sanitizer and exposure time. When the 11 cultures were exposed to individual sanitizers and mixtures of them in three replications, 2% formic acid and 1% formic plus 1% acetic acid were most effective, destroying 84 and 73%, respectively, of the test cultures. Three microorganisms that were refractory to 2% acetic acid were usually killed by 2% formic acid. Addition of ascorbic acid to the sanitizer to reduce oxidation of the meat pigments resulted in lowered microbial counts. Color was not affected by addition of 1% ascorbic acid. With added 5% ascorbic acid, discoloration was noticeable but not extensive. There was no effect of pH on microbial destruction at the concentration of acid used. However, more microorganisms were killed as time of exposure to the acid was increased.

Sanitizers are used in the food industry to decontaminate food contact surfaces. They can also be used to kill microorganisms on solid foods such as meat.

Anderson et al. (*2*) tested hypochlorite (200-250 ppm), acetic acid (4%) and a quaternary ammonium compound (3.78 mg/ml) on beef surfaces and found that counts made 48 h after sanitizing had increased by 0.53 and decreased by 1.79 and 0.03 logs, respectively. Further work by the same group (*1,3*) demonstrated that 3% acetic acid was a highly effective sanitizer for beef. It produced an average decrease of 2.55 in log count 48 h after sanitizing and increased shelf life of refrigerated meat 18-21 days, as measured by bacterial plate counts.

Several other researchers (*5,9,11,12,13*) have also examined the destruction of microorganisms on meat by various sanitizing sprays. Khan and Katamay demonstrated that short-chain fatty acids inhibited salmonellae in meat and bone meal (*8*). The recurring problem in most of these studies was surface discoloration of the meat by the acid sanitizer.

The purpose of the present research was to determine the bactericidal efficacies of selected short-chain fatty acids against microorganisms isolated from beef. A secondary objective was to determine the effects of the sanitizers and ascorbic acid on meat color.

MATERIALS AND METHODS

Materials

Nine of the cultures used were isolated from four fresh chuck steaks that had been sprayed with 3% acetic acid. They were *Streptococcus* sp. S6, *Micrococcus* sp. P, three film yeasts (Y_1, Y_2 Y_3) which reproduced by budding, a sporogenous bottom yeast (Y_4), two coliforms (C7 and C8) and a green pigment-producing pseudomonad (P27). Also used were a lipolytic culture of *Enterobacter aerogenes* (AL) and a yellow pigmented, slime-producing pseudomonad (P28) isolated from milk.

Sanitizers tested were potassium sorbate and formic, acetic and propionic acids. Ascorbic acid was used in the experiment on prevention of discoloration. All chemicals used were of purified or certified (Fisher Scientific Co., St. Louis, MO) grade. Solutions were prepared with tap water and used at 21 to 25 C. The recovery medium for the bacteria was trypticase soy broth and that for the yeasts was yeast-malt extract broth. Each contained 30 μg of bromthymol blue/l as a pH indicator.

Tissue culture plates with 24 wells each (Falcon Plastics, Oxnard, CA) were used in the screening experiments (termed the well test). A multiple transfer device was fashioned from six inoculating loops soldered 1.3 cm apart onto and perpendicular to the handle of an inoculating needle. Quantities of liquid transferred by the loops averaged 2.18 ± 0.06 μl.

Sanitizers were sprayed onto the meat with nozzle No. 5008 (Spraying Systems, Wheaton, IL) at a pressure of 14 kg/cm². The pH of each sanitizer was measured potentiometrically.

Methods

In each of three replications of the well test, the 11 test organisms were exposed individually to 1, 2 and 3% acetic acid solutions. Each solution was tested at pH 1.0, pH 2.0 and its normal (unadjusted) pH. Hydrochloric acid (HCl, 3N) was used to lower the pH. One milliliter of each sanitizer was measured into individual wells of tissue culture plates. The multiple loop device was used to transfer $2.2 \pm 0.6 \times 10^5$ organisms from 18-h-old broth cultures into the test solution. After 1, 5, 10 and 20 min, 2.18 μl of broth, containing 480 ± 130 cells (viable and non-viable) was transferred from the inoculated sanitizer to 1 ml of recovery broth (TSB) in wells of tissue culture plates. In addition, recovery broths in wells were inoculated with unexposed cultures for positive controls. Negative controls were made by transferring uninoculated sanitizer to recovery broths. Development of turbidity and a change in color of the bromthymol blue indicated that the sanitizer failed to kill all the microorganisms inoculated into it. Thus the test measured viability or nonviability of test cultures treated with the sanitizers.

In a second experiment, which was replicated three times, cultures were exposed to solutions in the same manner as in the first experiment. Exposure time was 60 sec. Each solution was tested at its normal pH and at pH 2.0. Any solution with a normal pH below 2.0 was tested only at that pH. Solutions containing potassium sorbate were also

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[1] *Contribution from the University of Missouri Agricultural Experiment Station. Journal Series No. 8304.*

[2] *Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*

[3] *The purpose of this paper is to report research findings. The U.S. Department of Agriculture does not suggest or recommend that these chemicals be utilized as antimicrobial agents or color enhancers on beef.*

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tested at their normal pH only, since lowering the pH caused sorbic acid to precipitate from solution. Wells with recovery broth were checked for growth after 24 and 36 h of incubation at 32 C.

After the second experiment, *E. aerogenes* AL, the film yeast Y_1 and *Micrococcus* sp. P were selected for further testing, since these three were the most resistant in previous tests.

Each of the three organisms was tested against a total of 16 acids and combinations of acids at concentrations of 1% and 2%. The exposure time for *Enterobacter* and *Micrococcus* cultures was 60 sec and those for the yeast were 60 sec and 10 min.

Ten strips of fresh frozen meat were thawed for each of two replications of a sanitizing experiment. For uniform microbial distribution on the meat, each surface to be treated was exposed to all others by rubbing it against each of the other surfaces (*4*). Samples were randomly paired, and each pair was placed on a holding frame by the method of Anderson et al. (*3*). Four samples (discs 2.54 cm in diameter and 3 mm thick) were removed from each pair (two from each piece of meat) and blended for 60 sec in 99 ml of sterile diluent. Plates were prepared and colonies counted according to the *Compendium of Methods for the Microbiological Examination of Foods* (*15*) except that incubation was at 28 C for 72 h.

Each pair of meat strips was subjected to one of the following treatments: no sanitizer (control), 3% acetic acid, 2% formic acid, 1% formic acid, and 0.5% acetic acid + 0.25% formic acid + 0.25% propionic acid. Conditions of application were the same as those used by Anderson et al, (*3*), namely 14 kg/cm² pressure, 6.8 l/min volume and 2 cm/sec speed of meat travel under the spray. After treatment, each meat strip was held with the long axis vertical and allowed to drain for 1 min. Four samples were removed from each pair, blended and plated as above. The meat was stored at 7 C and sampled again after 7 days.

In a second meat spraying experiment, 2% formic acid + 1% ascorbic acid, 2% formic acid + 5% ascorbic acid, and 2% formic acid alone were used as sanitizers. Conditions of applications were the same as in the first experiment.

Concentrations of sanitizers were based on earlier work done by Anderson et al. (*1,2,3*). Data were analyzed by analysis of variance (*14*) and, where variance was significant, treatment means were subjected to Duncan's multiple range test (*7*).

RESULTS AND DISCUSSION

The possibility that outgrowth of the test organisms in recovery broth could have been prevented by carryover of sanitizer was considered. The average amount of sanitizer transferred to recovery broth was 2.18 µl. When sanitizer concentration was 3% (30,000 mg/l), this would have resulted in a concentration of 65 mg/l in the recovery broth. The concentration at two standard deviations above the mean would have been 100 mg/l. Therefore, it is highly unlikely that cultures were inhibited in the recovery broths. Furthermore, the pH of the broth would have caused ionization of the acid in the recovery broth.

In the first experiment, exposure to acetic acid for 5, 10 and 20 min killed no more viable cultures than did exposure for 1 min. For each time the percentage killed was within the range of 41 to 42%. Therefore, data for all exposure times were combined in Table 1. These data demonstrated that pH was important. However, the large amount of HCl (approximately 2.5% of 3 N HCl) that had to be added to bring the pH to 1.0 was considered to be impractical to use. In this experiment, reducing the pH to 2.0 from the normal pH of the unadjusted acetic acid improved the efficacy of both the 2 and 3% solutions. However, in a second experiment the 13% greater kill by 2% acetic acid at pH 2.0 vs pH. 2.7 (normal pH) was not statistically significant (Table 2). The most effective of the sanitizers used in this experiment was the combination of 1% acetic + 1% formic acids. However, there was no significant difference in their activity at the normal pH and pH 2.0. The mixture of 2% acetic acid and 0.1% sorbate killed 60% of the cultures whereas 2% acetic acid alone destroyed 58%. It is unlikely, therefore that any contribution was made by sorbate. Sorbate is a fungistatic agent, and, since only bactericidal and fungicidal activities were measured in the experiment, the effect of sorbate could not be effectively determined. A 2% sorbate solution killed 12% of the cultures. Overall, the treatments did not show a significant pH effect when percentages of cultures killed at pH 2.0 and at normal pH's were compared. This result paralleled findings by Reynolds and Carpenter (*12*). They used phosphoric acid to lower pH of an acetic/propionic acid mixture and observed no increase in antimicrobial activity; there was, however, extensive surface discoloration of the meat which correlated with pH. Daly et al. (*6*) concluded from their work that formic acid inhibited *Staphylococcus* and that pH was inversely proportional to magnitude of antimicrobial effect. Since concentrations of acid used by

TABLE 1. *Percentages of 11 cultures made nonviable by acetic acid at three concentrations and at three pH levels.*[1]

pH	Percentage 1%	2%	3%
1.0	49	64	70
2.0	23	36	48
Initial pH	21[2]	28[3]	27[4]

[1]Combined data from exposures for 1, 5, 10 and 20 min with 11 cultures × 4 times × 3 reps = 132 observations per concentration per pH.
[2]pH. 3.2.
[3]pH. 2.7.
[4]pH 2.4.

TABLE 2. *Percentages of test cultures made nonviable by selected fatty acids at pH 2.0 and their normal pH as determined by the well test.*

Acid solution	Percentage[1] of cultures not viable pH 2.0	Normal pH[2]	
1% Acetic + 1% formic	87[a]	73[ab]	(2.1)
1% Acetic + 1% propionic	60[bcd]	55[bcd]	(2.8)
2% Acetic + 0.1% sorbate	*	60[bcd]	(4.1)
1% Acetic + 0.5% formic	60[bcd]	60[bcd]	(2.2)
1% Acetic + 0.5% formic + 0.1% sorbate	*	65[bc]	(3.0)
2% Acetic	58[bcd]	45[cd]	(2.7)
2% Formic	*	84[a]	(1.93)
2% Propionic	73[ab]	55[bcd]	(2.6)
2% Sorbate	*	12[e]	(7.1)
0.5% Acetic + 0.5% propionic + 0.5% formic + 0.1% sorbate	*	50[c]	(3.2)

[1]Three replications with 11 organisms per treatment.
[2]Normal pH in parenthesis.
[abcde]Values with a common superscript are not significantly different ($P < 0.05$).
*No test because initial pH was below 2.0 or low pH precipitated sorbate.

Daly et al. were low, increases in the H^+ concentration (lowering of pH) would have resulted in a shift to more undissociated acid, the form which has the antimicrobial activity. It is probable that concentrations used in the present research were high enough that the inverse relations of pH and antimicrobial activity were no longer operative. Also, meat has a high buffering effect and would tend to minimize pH differences at the meat surface.

After the second experiment, three organisms that were particularly resistant were selected for further testing (Table 3). These were the film yeast Y_1, *E. aerogenes* AL and *Micrococcus* sp. P.

Table 4 shows results from testing with *Enterobacter* and *Micrococcus*. Neither organism survived for 60 sec in 1% formic acid, nor did the *Micrococcus* survive in sanitizer containing 0.5% acetic acid + 0.25% formic acid + 0.25% propionic acid. *Enterobacter* was slightly more resistant than *Micrococcus* to the treatments.

Results of tests with 2% concentrations of the sanitizers are presented in Table 5. Although 2% formic acid and 1.5% acetic acid + 0.5% formic acid appeared to be most effective, percentages of cultures destroyed were not significantly different from the percentages destroyed by six other sanitizers. *Enterobacter* again was the more resistant organism.

TABLE 3. *Percentages of eleven cultures made nonviable by seventeen sanitizers in three replications of the well test.*

Culture	Percent not viable
Yeast-Y_1	4
Enterobacter-AL	14
Micrococcus-P	18
Yeast-Y_2	55
Yeast-Y_3	71
Yeast-Y_4	78
Streptococcus-S6	80
Pseudomonas-P28	84
Coliform-C8	84
Pseudomonas-P27	90
Coliform-C7	90

TABLE 4. *Percentages of* Enterobacter aerogenes *AL and* Micrococcu *sp. P made nonviable by fatty acids in 1% concentrations.*

Acid(s)[2]	%	Percentages not viable[1] *Enterobacter*	*Micrococcus*
F	1.0	100^{a}	100^{a}
A	1.0	0^{e}	10^{f}
P	1.0	0^{e}	15^{cef}
A + P	.5 + .5	0^{e}	0^{f}
A + F	.5 + .5	10^{d}	50^{bce}
P + F	.5 + .5	0^{e}	10^{f}
F + A	.75 + .25	35^{c}	50^{bce}
F + P	.75 + .25	65^{bc}	60^{b}
A + F	.75 + .25	0^{e}	0^{f}
A + P	.75 + .25	0^{e}	25^{bcef}
P + F	.75 + .25	0^{e}	15^{ef}
P + A	.75 + .25	0^{e}	0^{f}
F + A + P	.34 + .34 + .34	10^{d}	0^{f}
F + A + P	.50 + .25 + .25	35^{d}	65^{b}
P + A + F	.50 + .25 + .25	50^{bc}	60^{b}
A + P + F	.50 + .25 + .25	80^{bc}	100^{a}

[1]Three replications with 4 trials/treatment = 12 wells/culture/acid(s).
[2]F = formic, A = acetic, P = propionic.
[abcdef]Values with a common superscript within a column are not significantly different ($P < 0.05$).

TABLE 5. *Percentages of* Enterobacter aerogenes *AL and* Micrococcus *sp. P made nonviable by fatty acids in 2% concentrations.*

Acid(s)[2]	%	Percentages not viable[1] *Enterobacter*	*Micrococcus*
F	2.0	100^{a}	85^{a}
A	2.0	0^{c}	0^{d}
P	2.0	0^{c}	10^{d}
A + P	1.0 + 1.0	0^{c}	10^{d}
A + F	1.0 + 1.0	15^{c}	15^{cd}
P + F	1.0 + 1.0	60^{b}	15^{cd}
F + A	1.5 + 0.5	90^{ab}	65^{ab}
F + P	1.5 + 0.5	100^{a}	85^{a}
A + F	1.5 + 0.5	0^{c}	85^{a}
A + P	1.5 + 0.5	10^{c}	85^{a}
P + F	1.5 + 0.5	25^{c}	0^{d}
P + A	1.5 + 0.5	65^{b}	0^{d}
F + A + P	0.67 + 0.67 + 0.67	85^{ab}	35^{bc}
F + A + P	1.0 + 0.5 + 0.5	90^{ab}	40^{bc}
P + A + F	1.0 + 0.5 + 0.5	90^{ab}	65^{b}
A + P + F	1.0 + 0.5 + 0.5	90^{ab}	85^{a}

[1]Three replications with 4 trials/treatment = 12 wells/culture/acid(s).
[2]F = formic, A = acetic, P = propionic.
[abcd]Values with a common superscript within a column are not significantly different ($P < 0.05$).

No sanitizer in the 1% concentration completely inactivated all the cells of yeast Y_1. Therefore, Y_1 was tested against a 2% concentration of the sanitizers with exposure times of 60 sec and 10 min. The most effective sanitizer was 2% formic acid (Table 6). The 10-min exposure averaged over all sanitizers afforded 16% more destruction than did the 60-sec exposure, showing that extending the exposure time caused additional loss of viability ($P < 0.05$).

The most effective sanitizer in the meat-spraying experiment (Table 7) was 2% formic acid, which gave a log count difference (log count after treatment - log count before treatment) of -1.56 immediately after sanitizing. Counts of sanitized samples stored for 7 days at 7 C increased to only 0.92 log above counts made before sanitizing. This result supported earlier work by Kulshrestha and Marth (*9*), who tested several fatty acids

TABLE 6. *Percentages of tests in which sanitizers failed to kill yeast Y_1 in a total of 12 exposures per time per sanitizer.*

Acid(s)[2]	Percentages not viable[1] 60 sec	10 min
2% F	65^{a}	85^{a}
2% A	10^{b}	0^{d}
2% P	0^{b}	0^{d}
1% A + 1% P	10^{b}	0^{d}
1% F + 1% A	10^{b}	40^{bc}
1% F + 1% P	15^{b}	35^{bc}
1.5% F + 0.5% A	10^{b}	15^{cd}
1.5% F + 0.5% P	10^{b}	50^{b}
1.5% A + 0.5% F	10^{b}	25^{bcd}
1.5% A + 0.5% P	15^{b}	0^{d}
1.5% P + 0.5% A	10^{b}	0^{d}
1.5% P + 0.5% F	10^{b}	0^{d}
0.67% F + 0.67% + 0.67% P	0^{b}	25^{bcd}
1% A + 0.5% F + 0.5% P	10^{b}	25^{bcd}
1% F + 0.5% A + 0.5% P	0^{b}	10^{cd}
1% P + 0.5% F + 0.5% A	0^{b}	0^{d}

[1]Three replications with 4 trials/treatment = 12 wells/culture/acid(s).
[2]F = formic, A = acetic, P = propionic.
[abcd]Values with a common superscript within a column are not significantly different ($P < 0.05$).

TABLE 7. *Changes in viable counts resulting from sanitizing meat with fatty acids and storing the meat for 7 days at 7 C.*

Sanitizer	Changes in viable counts[1] Immediately after treatment[2]	After storage for 7 days at 7 C[2]
3% Acetic acid	-0.89^{b}	$+1.10^{a}$
2% Formic acid	-1.56^{a}	$+0.92^{a}$
1% Formic acid	-0.66^{c}	$+2.04^{b}$
0.5% Acetic + 0.25% formic + 0.25% propionic	-0.76^{bc}	$+2.24^{bc}$
Unsprayed control	0^{d}	$+4.66^{d}$

[1]Changes in viable counts are the log number of microorganisms counted after treatment minus the log number initially present.
[2]N = three replications with two strips of meat per treatment = 6.
[abcd]Values with the same superscript within a column are not significantly different ($P < 0.05$).

and found formic to be the most inhibitory to *Escherichia coli* and *Salmonella typhimurium.*

Although 3% acetic acid produced only about half the initial reduction in count as did 2% formic acid, counts of samples treated with 2% formic and 3% acetic acids were not significantly different when compared on the seventh day of storage. Compared with the above treatments, fewer organisms were killed by 1% formic acid and the mixture of 0.5% acetic acid + 0.25% formic acid + 0.25% propionic acid (Table 7). Counts of samples treated with 1% of the acids had increased by more than 2 logs after 7 days of storage but remained more than 2 logs less than the count for the unsprayed control. The control (unsprayed) meat increased in count logarithmically by 4.66 logs during storage. This value represents slightly more than 15 generations. Assuming a plate count of 10^8 represents spoilage, the unsprayed (control) meat spoiled within 7 days.

Normally, meat in transit would be held at about 3 C, but 7 C was used in this research to represent an accidental situation in which storage temperatures might rise above 3 C.

Meat sprayed with sanitizers containing formic acid began to turn brown within a few seconds after spraying and darkened further during storage. Discoloration appeared to be proportional to concentration. It was presumed that the meat pigment, myoglobin, had been oxidized to metmyoglobin. Presumably, the low pH of the sanitizer caused the ferrous ion in the porphyrin ring of the myoglobin molecule to be oxidized to the ferric ion.

Ascorbic acid was then added in 1 and 5% concentrations to the 2% formic acid in an effort to prevent oxidation of the myoglobin. After 7 days at 7 C, the meat treated with 2% formic acid + 1% ascorbic acid had a log count difference of only 1.1 compared with its initial count, but its color was about the same as that of meat sprayed with 2% formic acid alone. When the formic acid sanitizer was used with 5% ascorbic acid, discoloration was only slight and antimicrobial efficacy was enhanced. Meat treated with sanitizer alone had a log count difference of 0.92 after 7 days of storage, whereas that treated with sanitizer containing 5% ascorbic acid had a log count difference of 0.63 after storage.

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Bacteriological Assessment of Machine-Picked Meat of the Blue Crab[1]

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ABSTRACT

The bacterial quality of meat of the blue crab, *Callinectes sapidus*, picked by the "Quik-Pik" machine was investigated. Microbiological analyses were made on samples taken at each step throughout the machine-pick operation to determine the effect of operating procedures on the bacterial content of the finished product. Liquid from the cooked crab cores collected in crevices on the machine head, producing high levels of bacterial growth that was inaccessible to the sanitizing agent. Vibration of the machine head during operation created an aerosol which contaminated the fresh picked meat as it dropped to collection trays or the conveyor belt. The aerosol was the major source of contamination, producing counts well in excess of 100,000/g in the finished product. When the machine was dried with forced hot air, after sanitizing and before machine operation, the counts dropped dramatically to levels well below the 100,000/g limit. The bacterial quality of machine-picked crabmeat using the dry sanitized machine compared favorably to hand-picked meat.

During its nearly 100 years of existence the blue crab industry has changed very little. Current methods of harvesting have been used for years and hand-picked fresh crabmeat still constitutes the major product from the crab packing houses. Some years ago foresighted members of the industry recognized the need for mechanization brought about by increasing labor costs and a dwindling supply of new pickers. Considerable effort has gone into the development of machines, primarily by the industry itself with some assistance from government and the academic community, for washing and preparing cores and for extracting the meat from the prepared cores. The core is the central body portion remaining after the claws, legs, swimming fins, back, mouth parts and viscera have been removed.

A number of prototype models have been developed, and discarded for reasons such as cost, complexity of machine design with reference to cleaning and sanitizing, and unsatisfactory quality of the product (texture, taste, smell or appearance). Several are still under development and have real promise of fulfilling the industry's needs. The principles of meat extraction employed include: removal of the meat by vacuum (Sea Life Automation Corporation, Goldsboro, Md.) squeezing the meat from the legs and bodies with rollers (*4*), throwing the meat from the cores by centrifugal force (*7*), shaking the meat from the core by vibration (*2*) or crushing cores in a hammer mill and separating the meat from shell by brine flotation (the Harris Machine). To date, no machine produces crab meat of quality equal to hand-picked meat with appearance as a major quality factor. Backfin lump meat, the premium crabmeat product, is sacrificed unless removed from the cores by hand before machine-picking.

One of the most recent models currently of interest to the industry is the "Quik-Pik" (*6*) machine developed by the Sea Savory Co.[a], composed of a group of businessmen from the crabmeat industry. The Quik-Pik machine is relatively simple in design and, as its name implies, has the potential of producing upwards of 150 lbs. of meat per hour (picks 24 cores in 6 sec). The machine was placed in operation on a trial basis during the 1978 season.

This paper describes the microbiological assessment of the Quik-Pik process from whole cooked crabs to the finished, packaged product. The plants using the machine retained their hand-pick operations which permitted a comparison of the bacterial quality of the two crabmeat products.

In Maryland, the bacteriological limits for fresh crabmeat are 36 *Escherichia coli* most probable number (MPN) per 100 g and an aerobic plate count (APC) of 100,000 per gram. Meat with values in excess of either value is subject to seizure and condemnation (*1*). These limits were used to assess the bacteriological quality of both the machine and hand-picked products.

MATERIALS AND METHODS

Machine-pick operation

Crabs were cooked in the afternoon in a steam retort at 250 F for 12 min, held at room temperature until cool and moved to the cold room for overnight storage. Crab cores were prepared by hand for machine picking by removing the back (carapace), legs, swimming fins and claws. The viscera were scraped from the abdominal cavity and the cores washed with jets of cold water. Washed cores were either reheated and machine-picked immediately or returned to the cold room for heating and picking in the afternoon. Reheating cores before machine picking facilitated meat removal and prevented excessive shattering of the shell, resulting in a high shell content in the meat.

The Quik-Pik machine relies on vibration to remove meat from prepared cores. Cores are placed with viscera side down on racks, 24 cores per rack, and placed in the machine. The bladder inflates to hold cores in place and racks oscillate 3/8 inch about 70 times per second for 6 sec (*2*). Meat drops from the core cavity through openings in racks

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onto sanitized trays or a conveyor belt, the few shell fragments that may break off from the core are removed by hand and the meat is packaged.

The Quik-Pik machines evaluated were in two separate plants. The basic machine in each plant was the same. However, the process steps differed slightly. The two methods of operation are described below.

Plant I. Just before operation, the machine was sanitized with a chlorine solution (HTH, 200 ppm of active chlorine) and dried using a forced hot air drier. Cores were prepared in the morning, loaded onto picking racks, washed, steam heated to 230 F and machine-picked immediately. Meat was collected on trays, deboned and packaged.

Plant II. Cores were prepared and washed in the morning and stored in the cold room. In the afternoon the machine was sanitized by the generous application of a commercial iodophor solution (Du Boise, 25 ppm) and placed in operation without drying. Cores were heated to 230 F in a steam retort, loaded onto picking racks and machine-picked. The meat was collected on trays or a conveyor belt, deboned and packaged.

The optimum core temperature to facilitate picking a practically bone-free product with an acceptable bacterial quality was investigated in Plant I by heating the cores in the steam retort for 1 min at retort temperatures from 175 to 230 F.

Samples taken at each step in the machine-pick process were analyzed for APC and total coliform and *E. coli* MPN's/100 g of meat. The APC provides a measure of the numbers of viable aerobic and facultative anaerobic heterotrophic bacteria per gram of sample (*3*). Composite crabmeat samples were taken from each plant's hand-picking room and from the hand-picking room of a third plant for comparison of the bacterial quality of the two products.

Media

Media (Difco) used were consistent with those used by the Maryland State Department of Health and Mental Hygiene for the microbiological analysis of crabmeat. Plate Count Agar (PCA) served as a plating medium for APC. Lauryl Tryptose Broth (LST), double and single strength, was used as the coliform presumptive test medium. Coliforms were confirmed with Brilliant Green Bile, 2% (BGB) and *Escherichia coli* were confirmed with EC Medium, Levine's EMB, and Simmons Citrate agar. Butterfield's buffered phosphate diluent at pH 7.2 served as dilution water for all analyses.

Sample collection and types

Samples were collected aseptically, placed in sterilized containers and stored in foamed plastic chests filled with crushed ice for transport to the laboratory. They were analyzed as soon as possible upon return to the laboratory and in no instance did the time between sample collection and analysis exceed 24 h.

Whole crab samples (5-6 cooked crabs per sample) were collected from: (*a*) cold storage, (*b*) tables in the hand-picking room and (*c*) just before core preparation. Core samples (4-5 cores per sample) were taken: (*a*) after washing, (*b*) after cold room storage and (*c*) after reheating just before picking. Each sample of whole crabs or cores was placed in a sterile 1/2 gal can with a press lid.

Crabmeat samples, consisting of 75-100 g of meat per sample, were collected: (*a*) from trays or conveyor belt just after machine-picking but before removal of shell fragments by the inspector and (*b*) from the packaged machine-picked meat product after removal of shell. "Caught" meat samples were taken by placing the sample container under the rack holding the cores during the machine vibrating operation through two shake cycles to catch the meat as it dropped from the cores. Composite crabmeat samples were taken in the hand-picking room by collecting, in one sterile container, several grams of meat from each picker. One-pound crabmeat cans (401 × 301) with snap-on lids served as containers for all crabmeat samples.

Sample analyses

Crabmeat. Fifty grams of meat were homogenized with 450 g of dilution water for 90 sec in a Waring Blender to prepare a 1:10 homogenate. Serial 10-fold dilutions were prepared through 1:10,000; the 1:100, 1:1,000 and 1:10,000 dilutions were plated on PCA, 1 ml per plate, two plates per dilution to give the APC. Plates were counted after 48 h of incubation at 35 C. For determination of total coliforms and *E. coli*, 10 ml of the 1:10 dilution were inoculated into three tubes of double-strength LST and 1 ml from the 1:10 and 1:100 dilutions were added to a single-strength LST, three tubes per dilution. The LST tubes were incubated at 35 C and examined at 24 and 48 h. Material from positive tubes (gas production) was transferred to BGB and EC medium. After 24 and 48 h at 35 C BGB cultures were examined for gas production. Gas production in BGB confirmed the presence of coliforms. The EC tubes were incubated at 44.5 C in a covered, circulating water bath. After 24 and 48 h bacteria in those tubes showing gas were streaked on EMB agar to select for *E. coli*. The EMB plates were incubated at 35 C for 24 h and colonies typical for *E. coli* (small, round black colonies with a greenish metallic sheen) were picked and streaked on Simmons citrate slants and incubated at 35 C for 48 h. If typical colonies were not present, two colonies judged most likely to be *E. coli* were picked and transferred to Simmons citrate slants. Those EC tubes that contained gas and yielded typical, or near typical, colonies on EMB that failed to grow on Simmons citrate agar, were considered positive for *E. coli*. The total coliforms and *E. coli* were reported as the most probable number (MPN) per 100 g of sample.

Whole crab and crab cores. Procedures for whole crab and crab core analyses followed those described by Ward and Tatro (*5*). One part of whole crab or core was blended for 2 min with 2 parts of dilution water. Thirty ml of the 1:3 homogenate were diluted to a concentration of 1:10 by addition of 70 ml of dilution water. The subsequent procedures followed those outlined for crabmeat.

RESULTS AND DISCUSSION

The APC and total coliform and *E. coli* MPN's for the machine-pick operation in Plants I and II are shown in Tables 1 and 2, respectively. The data reported represent the mean values obtained from duplicate samples collected at each sampling point over a 17-week period from 8/15/78 to 12/6/78. The two processes compare quite favorably up through the "caught" meat samples. Though stored cores prepared in the morning showed an increase in bacterial content, the counts dropped to comparable levels after heating to 230 F. From that point on, the bacterial levels increased dramatically in the Plant II operation. Open petri dishes of PCA placed on the conveyor belt beneath the head of the machine through one shake cycle without crab cores were crowded with colonies, and a count was not possible. Apparently the vibration produced a heavily contaminated aerosol that was thrown down onto surfaces below contaminating the meat. This is emphasized by the "caught" meat samples which were collected immediately below the rack before the meat was exposed to the contaminating aerosol. After 0.5 to 0.75 h of operation, the machine head had

TABLE 1. *Bacteriological assessment of Machine-Pick process in Plant I.*

Sample type (Number Collected)	APC/g[1]	Total Coliform (MPN/100 g)	*E. coli* (MNP/100 g)
Whole crab (13)	59,000	320	< 30
Cores unwashed (5)	46,000	930	< 30
Washed cores (5)	24,000	680	< 30
Heated cores (5)	< 3,000[2]	< 30[3]	< 30
Caught meat (5)	< 3,000	46	< 30
Tray meat (15)	17,000	350	< 30
Packaged meat (15)	30,000	3900	< 30

[1]Arithmetical mean.
[2]With the APC procedures used 3000 was the lowest count considered accurate.
[3]The lowest level of total coliforms or *E. coli* detectable was 30/100 g.

cleansed itself of much of the contaminated liquid and the counts in the meat began to drop. It was thought that liquid from the crab cores remained on the surfaces of the head of the machine, and collected in crevices where different components were bolted together. This liquid produced high bacterial growth overnight that was inaccessible to a sanitizer. An attempt was made to seal the crevices, but there was little or no improvement in bacterial quality of the meat. The problem was avoided in Plant I by drying the machine before operation, thereby removing the offending liquid.

The effect of core temperature on the APC of machine-picked crabmeat in Plant I is shown in Table 3. Though all temperatures produced meat with an acceptable count, the range of counts narrowed with increasing temperature, as expected. The temperature investigations were made during December when counts on raw crabs are naturally lower. Dormant crabs, harvested by dredging, were used. These crabs contrasted with free swimming crabs caught with crabpots and trotlines, and used during most of the season. Therefore, results should be applied conservatively to summertime operation when core temperatures from 195 to 230 F might give more consistent and predictable counts. The low count routinely achieved with 230 F is very attractive to the microbiologist. However, from the processors standpoint, other factors of quality such as texture or moisture may favor a lower temperature.

A comparison of the bacterial quality of machine-picked and hand-picked meat is shown in Table 4. The machine-picked meat produced by Plant I compared very favorably to the hand-picked products from all three plants. It should be mentioned that the machine samples at the higher end of the range were collected later in the day after 4-6 h of operation without any shut down time for machine cleanup or sanitation. The high counts seen in the machine-picked product from Plant II were solely due to the difference in machine preparation and not laxity of sanitary procedures. The low bacterial levels in the hand-picked product in this plant throughout the sampling period attest to the high standards of sanitation observed.

TABLE 3. *Aerobic plate count of machine-picked crabmeat from crab cores heated in steam retort to various temperatures.*

Maximum retort temperature (F)	APC/g		
	Range	Mean[1]	Standard Deviation
175	11,000-89,000	53,000	25,987
185	10,000-25,000	17,000	5,278
195	10,000-21,000	14,000	4,166
205	8,500-17,000	12,000	3,177
230	3,000-10,000	5,500	2,231

[1]Numerical average of eight trials at each temperature.

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TABLE 2. *Bacteriological assessment of Machine-Pick process in Plant II.*

Sample type (Number collected)	APC/g		Total Coliform (MPN/100 g)		*E. coli* (MPN/100 g)	
	A[1]	B[2]	A[1]	B[2]	A[1]	B[2]
Whole Crab (6)	48,000		91		< 30	
Cores, Unwashed (5)	19,000		930		< 30	
Washed Cores (5)	9,500		460		< 30	
Stored Cores (5)	45,000		7,000		< 30	
Heated Cores (5)	3,100		670		< 30	
Caught Meat (10)	< 3,000	< 3,000	4,600	230	< 30	< 30
Tray Meat (15)	92,000	28,000	> 24,000	2,400	< 30	< 30
Belt Meat (15)	300,000	11,000	> 24,000	2,400	< 30	< 30
Packaged Meat (15)	860,000	100,000	> 24,000	> 24,000	< 30	< 30

[1]Meat samples taken during 1st 5 min of operation.
[2]Meat samples taken after 1/2 - 3/4 h of operation.

TABLE 4. *Comparison of bacterial quality of machine-picked to hand-picked crabmeat.*

Plant	Operation	SPC/g		Total coliform (MPN/100 g)		*E. coli* (MPN/100 g)
		Range	Mean	Range	Mean	
I	Machine[1]	8,000- 90,000	30,000	230- > 24,000	4,600	< 30
	Hand[2]	5,000- 260,000	78,000	230- > 24,000	6,300	[3]
II	Machine[1]	100,000-2,000,000	860,000	2,400- > 24,000	11,000	< 30
	Hand[2]	14,000- 30,000	22,000	91- 930	430	< 30
III	Hand[2]	20,000- 57,000	37,000	930- 2,400	1,600	< 30

[1]Packaged meat.
[2]13 samples collected from Plant I, and 12 each from Plant II and III.
[3]Two samples of 13 contained 36 and 91 *E. coli* MPN/100 g.

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Comparison of Code Date Reliability for Freshly Bottled Whole, Lowfat and Nonfat Fluid Milk

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ABSTRACT

Whole, lowfat, and nonfat fluid milk samples were collected from dairy plants on the day of bottling and each set of the three types of milk was processed from the same raw milk supply. Bacterial analyses for total aerobic count and for specific degradative types were made immediately after collection. Samples were also stored at 1.7, 5.6 and 10.0 C to test for flavor deterioration. Total count of aerobic bacteria and of specific degradative types did not differ significantly among the three types of milk. Organoleptic analysis was made periodically to determine milk quality (flavor score) and defect (flavor). At any of the storage temperatures the keeping quality (days required from bottling to reach a flavor score of < 36) was unrelated to the length of time between bottling and last day of sale (code date) assigned by the processor. At 5.6- and 10.0-C storage, more whole milk samples were criticized for more serious flavor defects (e.g. putrid) than for the less serious ones (e.g. lacking freshness) found in the lower fat milks. Keeping quality of all three types of milks at 1.7- and 5.6-C storage could be predicted from keeping quality determined at 10.0 C with equations previously developed for whole milk.

Recently we reported on the keeping quality of milk offered for retail sale in Connecticut (*2,3*). These studies were made on whole milk, i.e., milk with at least 3.25% of milkfat, no added solids but fortified with vitamin D. The samples were collected both at retail stores and directly from processing plants. We showed that the keeping quality (organoleptic acceptability) of the product at any storage temperature was unrelated to the processor's code date (last day product is to be sold). There was a significant correlation between keeping quality at 10.0-C storage and keeping quality at 1.7- and 5.6-C storage, suggesting a practical test to measure keeping quality at the lower storage temperatures.

Processors, at least in Connecticut, use the same code period (time between date of bottling and code date) for all three types of fluid milk, whole, lowfat and nonfat. Since our previous studies had shown the code date to be unrelated to actual keeping quality we investigated whether this same situation existed for the two lowfat milks. This study also allowed us to determine what flavor defects developed in these products and to assess the types of bacterial contaminants present.

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MATERIALS AND METHODS

Sampling

At eight intervals from January, 1977 through October, 1978, 42 samples of pasteurized whole, lowfat and nonfat milk were collected from six large dairies in Connecticut. All three products were collected at each dairy at the same time, reflecting that all the products were processed from the same supply of raw milk contained in a single storage tank and bottled on the same day. If samples were collected from the filler, all samples were taken from the same filling valve. No samples were collected on Mondays since such milk could have been stored over a weekend. Both actual inspection and close questioning assured that all milk was processed from the same raw supply. Each lot consisted of four samples collected in original containers which were placed in ice during transport to the laboratory. One subsample was used for microbiological analysis and the other three subsamples for organoleptic analysis.

Organoleptic analysis

Subsamples were stored at 1.7, 5.6 and 10.0 C. Organoleptic analysis was made as described previously (*1,2,3*) by at least two trained persons according to procedures recommended by the American Dairy Science Association as modified for use in the Connecticut Milk Flavor Improvement Program.

Microbial analysis

Microbial tests were made initially on each sample as it arrived in the laboratory. The method of plating and the media used have been described (*2,3,4*).

Statistical analysis

All data were analyzed statistically using SPSS computer programs (*5*).

Definitions

"Code date" is the date marked on the container designating the last day on which the product may be sold or offered for sale. "Code period" is the number of days between bottling and the code date. "Days to go bad" is the number of days from bottling until the flavor score dropped below 36. "Keeping quality" is the number of days from bottling that a sample remained organoleptically acceptable, i.e., with a flavor score of 36 or higher.

RESULTS AND DISCUSSION

Keeping quality in relation to code date and storage temperature

Shown in Table 1 are data for actual keeping quality of 42 samples of whole, lowfat and nonfat milk collected at processing plants and all processed from the same supply of raw milk. The samples were stored at 1.7, 5.6 and 10.0 C. The average code period for all samples was 11.1 days. Thus at 1.7-C storage all samples exceeded the code period and at 5.6 C storage only the nonfat milk

failed to retain a satisfactory flavor to the end of the code period. At 10.0-C storage none of the milk retained an acceptable flavor to the time designated by the processor. There was no statistically significant difference between the keeping quality of the three types of milk at any of the storage temperatures. Further, for all three types of milk no significant correlation was found between the number of days for a sample to become unacceptable (reach a flavor score of less than 36) and the code period assigned by the processor, when the sample was stored at either 1.7, 5.6 or 10.0 C.

Previously we had calculated two regression equations to predict the keeping quality of whole milk stored at 1.7 and 5.6 C based on keeping quality at storage at 10.0 C (*3*). The formulae were:

$$\hat{Y}_{5.6\,C} = 0.44 + 1.68X_{10.0\,C} \text{ and } \hat{Y}_{1.7\,C} = 5.01 + 1.8X_{10.0\,C}$$

where X is the number of days required at 10.0-C storage until the flavor score dropped below 36 and $\hat{Y}$ is the predicted number of days to go bad at the other designated storage temperatures.

Using these equations estimated for whole milk, a prediction was made on the keeping quality of the whole, lowfat and nonfat milk tested in the present study (Table 1). In no instance was the mean prediction in error by more than 1 day. Thus the prediction equations based on whole milk appear from a practical standpoint to be satisfactory for milk with a lower fat content.

From the present data, equations were developed predicting days to go bad for each of the lowfat products. They differed little in overall predictive ability from the original equations developed for whole milk (*3*).

Bacterial groups found in whole, lowfat, and nonfat milk

The number of bacteria found in the freshly pasteurized milk is shown in Table 2. The number of aerobic bacteria is quite low as are the numbers of the other bacterial groups. No significant differences were found among populations of any bacterial group in the three types of milk. Further, data on the percentage of the total count for any bacterial group among the three types of milk examined show no trend in the destruction of any specific bacterial group able to excrete a specific degradative enzyme. Thus the amount of fat in the milk evidently has little effect on destruction by pasteurization of the types of degradative bacteria present in the raw milk.

Flavor of milk after storage

The flavor defect of each sample of whole, lowfat and nonfat milk was determined when it was no longer organoleptically acceptable (flavor score of below 36) (Table 3). On initial testing many of the lowfat and nonfat milks were criticized as having a vitamin-like flavor although the number of samples having a cooked flavor was also high. The vitamin-like flavor is attributable to the vitamin A fortification used in the lower fat milks but not used in the whole milk. The "burnt" flavor criticism of the milk is attributed to paper cartons overheated during sealing.

Most of the samples stored at 1.7 C were rejected for the generally non-specific criticism of lacking freshness. At the higher storage temperatures of 5.6 and 10.0 C, where mesophilic bacteria are more likely to grow, the more serious flavor defects such as putrid or curdled were evident, especially in the whole milk. The lower fat milks had the less serious defect of lacking freshness. No general pattern of flavor criticism among the three types of milk was noted. A comparison of the present data with observations of milk samples collected directly from processing plants in 1977 (*3*) showed similar results.

TABLE 1. *Actual and predicted keeping quality*[1] *of whole, lowfat and nonfat milk stored at 1.7, 5.6, and 10.0 C.*

	Keeping quality				
	Actual[2] no. days			Predicted no. days from 10.0-C storage	
Type of milk	1.7 C	5.6 C	10.0 C	1.7 C	5.6 C
Nonfat	16.7 ± 0.6[3]	10.9 ± 0.5	6.8 ± 0.3	17.3 ± 0.5	11.8 ± 0.5
Lowfat	17.4 ± 0.5	12.8 ± 0.7	6.9 ± 0.3	17.6 ± 0.6	12.1 ± 0.5
Whole	16.8 ± 0.4	12.9 ± 0.6	7.1 ± 0.4	17.8 ± 0.6	12.3 ± 0.6

[1]Based on the prediction equations for whole milk (3.25% fat minimum); $\hat{Y}_{1.7\,C} = 5.01 + 1.81X_{10.0\,C}$ and $\hat{Y}_{5.6\,C} = 0.44 + 1.68X_{10.0\,C}$ (see ref. *3*).
[2]Average code period for all samples was 11.1 days.
[3]Standard error of the mean.

TABLE 2. *Number of percentage of various types of bacteria in freshly bottled whole, lowfat and nonfat milk.*[1,2]

	Whole milk		Lowfat milk		Nonfat milk	
Bacterial group	No.[3]	%[4]	No.	%	No.	%
Total aerobic count	617	—	688	—	466	—
Protease producers	97	15.7	147	21.4	96	20.6
Lipase producers	266	43.1	392	57.0	285	61.2
Pseudomonads	99	16.0	135	19.6	102	21.9
Protease-producing pseudomonads	51	8.3	85	12.4	59	12.7
Lipase-producing pseudomonads	89	14.4	119	17.3	88	18.8
Acid producers	14	2.3	29	4.2	14	3.0

[1]14 samples of each type represented; samples collected from processing plant on day of bottling and all milk processed from same raw supply.
[2]t-test shows no significant difference between types of milk for the different bacterial groups shown.
[3]Number of colony forming units per ml of milk.
[4]Percent of total aerobic count.

TABLE 3. *Initial flavor at sampling (I) and flavor of three types of milk when no longer organoleptically acceptable after storage at 1.7, 5.6, or 10.0 C.*

Flavor	Whole milk				Lowfat milk				Nonfat milk			
	I	10.0 C	5.6 C	1.7 C	I	10.0 C	5.6 C	1.7 C	I	10.0 C	5.6 C	1.7 C
	(% of samples)				(% of samples)				(% of samples)			
Cooked	7.1											
Cooked & unclean	28.6											
Feed & unclean	64.3											
Feed					28.6				7.1			
Vitamin-like					49.9				78.6			
Cooked & feed					14.3				—			
Burnt					7.1				14.3			
Lacks freshness	—	35.7	28.6	100	—	78.6	42.9	100	—	71.4	50.0	85.7
Putrid/curdled	—	50.0	57.1	—	—	7.1	35.7	—	—	7.1	21.4	7.1
Unclean	—	7.1	—	—	—	—	—	—	—	—	—	—
Bitter	—	7.1	7.1	—	—	7.1	—	—	—	—	21.4	7.1
Fruity	—	—	7.1	—	—	7.1	14.3	—	—	—	—	—
Rancid	—	—	—	—	—	—	7.1	—	—	—	—	—
Avg. no. of days to become unacceptable	—	7.1	12.9	16.8	—	6.9	12.8	17.4	—	6.8	10.9	16.7

Samples collected in 1976 from retail stores (*2*), however, had more serious flavor defects.

The keeping quality of whole, lowfat and nonfat milk does not differ significantly at a given storage temperature. As we had shown previously for whole milk, the code period for lowfat and nonfat milk was also unrelated to the actual keeping quality. Bacterial populations in all three types of milk were as low as we had found in whole milk collected at processing plants. Clearly, high storage temperatures damaged keeping quality of all three kinds of milk. At 5.6 C whole and lowfat milk, on the average, remained acceptable approximately 1 day beyond the average code period for all samples, 11.1 days. However, nonfat milk stored at 5.6 C did not remain acceptable to the end of the code period. Since storage temperature in retail stores frequently exceeds 5.6 C (*2*), it is easy to see why milk would not retain an acceptable flavor through the last date of sale.

Most investigations of microbial quality and flavor quality or flavor deterioration have examined whole milk collected at dairies or at retail outlets. Few studies report on quality of lower fat milks. In the present study we have compared milk of different fat contents, all from the same dairy and all processed from the same raw milk supply and found no evidence that separate code periods need be used for different types of milk, nor did we find changes in the character of the microbial contaminants.

ACKNOWLEDGMENTS

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SMEDP-15, *con't from p. 245*

are described in the following paragraphs.

1. *Recovery of Injured Coliforms.* Procedures for allowing recovery of injured cells have involved overlay techniques and use of modifications of the bile agar medium. Collaborative studies are needed as well as comparative studies between the methods. (Dr. E. H. Marth, Department of Food Science, Babcock Hall, University of Wisconsin, Madison, WI 53706; phone: 608-263-2004).

2. *Rose Bengal-Chlortetracycline Hydrochloride Agar.* This medium has been shown to encourage growth of some yeasts and molds which are inhibited by acidified potato dextrose agar. The medium also limits colony spreading. A collaborative study is needed to confirm the advantages of this medium for recovery of yeasts and molds in dairy foods. (Dr. E. H. Marth, Department of Food Science, Babcock Hall, University of Wisconsin, Madison, WI 53706; phone: 608-263-2004).

3. *Hydrophobic Grid Membrane Filter Methods.* Recent improvements in membrane filter methods for microbiological examination of foods prevent colony spread and allow enumeration of a wider range of colony forming units. Coliform tests on dairy foods containing sucrose are possible and up to 5 g of dairy foods can be filtered through such membranes. Technique development and collaborative studies are suggested. (Dr. Gary H. Richardson, Department of Nutrition and Food Science, Utah State University, Logan, UT 84322; phone: 801-750-2120).

4. *Rapid Psychrotrophic Methods.* There is need for review, refinement, and miniaturization of

con't on p. 189

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Lead Content of Milk and Infant Formula

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ABSTRACT

A survey to determine the lead content of early-infant food sources was conducted in the District of Columbia. Samples were collected from various lots of national brands of infant formula and evaporated milk, cartons of nonfat dry milk, containers of homogenized cow's milk and human milk from volunteer mothers. Data indicate that the concentration of lead in infant formula, evaporated milk and nonfat dry milk exceeds that in fresh cow's milk and human milk.

Since the early 1970's breast-feeding has been increasing in American culture. No more than 18% of new mothers were breast-feeding their babies on leaving the hospital in 1966. By 1976, 33% of the mothers were breast-feeding one week postpartum (*1*).

As the number of women choosing to breast-feed has grown, environmental medicine specialists are concerned about the contamination of human milk with an increasing number of environmental chemicals such as polychlorinated biphenyls, polybrominated biphenyls, other lipophilic contaminants and heavy metals.

Although both dispersive and contained uses of many of these contaminants have been reduced, harmful chemicals still persist in the water contact cycle, in soil and in food sources of some regions.

Of the heavy metals, lead continues to elicit increasing attention as the debate sharpens over whether or not blood-lead levels below those associated with obvious symptoms have adverse effects on the brain.

In addition, there is growing recognition that elevated blood levels in children are no longer confined solely to those who live in high risk areas - the so-called lead belt which is characterized by substandard housing - but infants in standard affluent neighborhoods are also susceptible to the neurotoxic effects of lead at various doses.

Although lead-based paints and lead in the urban atmosphere are major sources of lead-induced stress on infants and young children, consumer goods also contribute to this overall burden. For example, milk is a major source of nutrients for infants and there is special concern for the contribution of this food to the total dietary intake.

Therefore a survey was conducted of the lead content of early-infant food sources in Washington, D.C. to (*a*) develop a reference frame for estimating the approximate dietary intake of lead of infants, and (*b*) to construct a data base for future determination of trends in the lead content of infant foods as well as for risk assessment.

METHODS

During 1978 and 1979 the following specimens were collected: (*a*) cans from various lots of national brands of infant formula and evaporated milk, (*b*) cartons of nonfat dry milk, (*c*) containers of homogenized cow's milk and (*d*) human milk from volunteer mothers who were regular visitors to local maternal and infant care clinics. These samples were collected in lead-free containers.

Approximately 25 ml of samples were weighed in a 200-ml beaker. The samples were evaporated to dryness and then charred in a low temperature asher. The charred product was then digested in nitric acid and sulfuric acid. Lead determinations were then made with the use of a Perkin-Elmer Model 603 atomic absorption spectrophotometer. Cow's milk and human milk were analyzed with the same equipment but with the Delves cup technique (*4,5*), by the method of addition.

RESULTS AND DISCUSSION

The lead concentrations in infant formula are shown in Table 1. Table 2 shows lead levels in other sources of milk in infant diets.

These data indicate that the concentration of lead in infant formula, evaporated milk and nonfat dry milk exceeds that in fresh cow's milk and human milk.

Our findings are in agreement with those of other studies. For example, Lamm et al. (*8*) found that in Connecticut skimmed milk, canned concentrate infant formula, and evaporated milk appeared to deliver a much larger lead dosage to infants than a diet of nonfat dry milk, homogenized cow's milk or human milk.

These data are additions to the base of knowledge necessary to establish (*a*) dose-effect (exposure-effect)

TABLE 1. *Lead content of canned infant formula.*

Brand number[a]	Number of samples	Concentration (μg /ml) Mean	Range
1	10	0.05	0.03-0.07
2	10	0.05	0.03-0.09
3	10	0.05	0.03-0.07
4	10	0.05	0.01-0.06
5	10	0.50	0.40-0.57
6	10	0.06	0.05-0.09
7	10	0.07	0.05-0.90
8	10	0.09	0.05-0.10
9	10	0.08	0.02-0.09
10	10	0.08	0.07-0.10
11	10	0.40	0.00-0.60
	Total 110		

[a]Different lots of the same products.

TABLE 2. *Concentration of lead in milk supplies.*

Type of milk			Concentration (μg /ml) Mean	Range
Evaporated	2	10	0.30	0.29-0.36
Homogenized cow's	2	10	0.01	0.01-0.02
Nonfat dry	2	10	0.53[a]	0.50-0.79
Human breast	20[b]	40	0.02	0.00-0.05
Total	26	Total 70		

[a]Microgram/gram.
[b]Number of donors.

relationships, (*b*) toxicity thresholds and consequently (*c*) permissible limits; all of which constitute a fundamental principle of prevention.

Indeed the potential harmful action inherent in a substance such as lead is manifest only when that substance comes into contact with a living biological system. A substance normally thought of as "harmless" will evoke a toxic response if added to a biological system in significant amounts.

However, an unsettled question in the lead exposure issue is the daily permissible intake of lead (DPI). King (*7*), who chaired at U.S. committee to establish a DPI, recommended 300 μg of elemented lead while Baltrop (*2*) proposed 93-133 μg per day. Lamm et al. (*8*) reported that although the lead intake in early infancy varies with the type and amount of diet, infants on canned formula or evaporated milk ingested lead in amounts measured in hundreds of micrograms per day. Thus it is deduced that infants may be at risk of accumulating lead from such milk diets.

Our experiences in the District of Columbia suggest that the attitude of the individual (mother, grandmother, father, etc.) who feeds the infant affects the volume of the product consumed. An interview of 40 mothers with infants between 40 and 50 days of age indicated that the median intake of formula was approximately 178 ml/kg/day. Brown (*3*) found a median of 168 ml/kg/day.

We have also found that the pattern of milk consumption and thus lead intake varies among income groups. During the first three months of life, canned formula is the most common source of milk in the infant diet; after 8 to 10 months, the vast majority of children drink cow's milk. The frequency of breast-feeding among mothers of middle-income groups is about 25-30% during the first month while it is approximately 10% among poorer women. Lower-income mothers usually have more social and economic problems to cope with and therefore rely to a large extent on the bottle because it is more convenient and less time consuming.

The popularity of breast-feeding also varies geographically. In southern California 50 to 60% of the mothers start out nursing their infants. But in one Philadelphia maternity center, 80 to 85% of mothers of all socioeconomic levels breast-feed their infants (*1*).

These patterns of consumption must be considered in estimating the probable dietary intake of lead during early infancy. For example, since human milk has less lead than formula or evaporated milk, the intake of lead by infants from middle-income families during the first few months of life is probably less than that of infants from poorer families.

Important also is the fact that the tissue-lead value at any given time reflects a complex balance among the exposure, the amount absorbed from both the lungs and the intestinal tract and that amount stored in the bones. In addition, damage to the respiratory tract caused by infection, liver or kidney defects and hematological disorders may play a part in the precise amount of lead to be found in a tissue.

Although the DPI for early infants has not been firmly established, the threshold for undue lead adsorption has been reduced from 40 μg per 100 ml of whole blood to 30 μg over the past three decades. This downward adjustment is consistent with new data on the effects of lower doses of the lead burden. For example, Piomelli (*12*) reported in 1977 that at blood-lead levels of 15 μg per deciliter there is an elevation of free erythrocyte protoporphyrin. Similarly, Hernberg (*6*) demonstrated the effects of low lead levels on the enzyme delta-amino-levulinic acid dehydratase and Nathanson (*9*) reported on lead induced-inhibition of brain adenylcyclase.

More recently Needleman et al. (*10*) studied the deficit in psychologic and classroom performance of children with elevated (58 children with high and 100 with low) dentine lead. They concluded that lead exposure at doses below those producing symptoms severe enough to be diagnosed clinically appears to be associated with neuropsychologic deficits that may interfere with classroom performance.

The neurotoxic properties of lead at high doses are so well known that a review here would approach impertinence. What is not entirely clear is whether or not blood-lead levels below those associated with obvious symptoms have adverse effects on the brain or other anatomical or physiological sites.

However, by definition a tissue has a pollutant burden whenever it contains an environmental residue greater than that needed for optimum growth and development. Since lead is not a dietary essential, any amount of this heavy metal in a tissue places a burden on the biological system. Thus while the lead content of infant foods is now lower than in earlier periods of commercial food production, continuing efforts are necessary to further reduce the potential pollutant burden on infants.

This is especially critical in view of the fact that lead poisoning has shifted from an industrial hazard to an environmental hazard with increasing exposure of pregnant females to combustion products of lead-containing fuel and other population-induced dispersion of lead. Moreover, studies of maternal-fetal tissues have provided evidence for accumulation and transplacental transfer of lead which can alter the development of the fetus (*11,13*).

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Effect of Thaw Conditions on Ground Beef [1]

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ABSTRACT

Three sampling periods were selected to acquire ground beef packages from two retail stores. Initially, samples were subjectively evaluated for color and overall appearance. Microbial load and taxonomy were determined and the samples were subsequently frozen. After frozen storage, one-half of the packages were thawed at 5 C for 4, 8, 24, 48 and 72 h, whereas the other samples were thawed at 25 C for the same periods. After thawing, samples were evaluated for the same characteristics as before freezing. Samples from each treatment were also evaluated for flavor, odor and overall acceptability. Appearance traits, taste and microbial load were affected ($P < .05$) by thaw time and temperature. Major groups of bacteria identified were: micrococci, *Pseudomonas*, streptococci, staphylococci, *Flavobacterium*, lactobacilli and coliforms. *Salmonella* and pathogenic *Staphylococcus* were not found. Those microorganisms which were identified were considered to be responsible for deterioration of appearance and taste attributes. Increased coliform growth at 25 C, especially after 8 h, suggests a need to thaw ground beef at refrigerated temperature to reduce public health concern. Results suggest that a proper thaw time and temperature to maintain acceptable appearance and taste of ground beef would be 24 h or less at refrigerated temperature.

Ground beef is one of the most versatile and widely accepted foods of the American diet. Over 75% of all households serve ground beef at least once a week, and it accounts for nearly one-half of all beef consumed. Consumption of this foodstuff through fast food outlets has increased during the past decade, and additional consumption through this market channel remains a strong possibility (*6*).

Since ground beef is a nutritious foodstuff, it can provide an environment conducive to microbial growth. Microorganisms are introduced by contamination of the meat surface from equipment, air, man and other sources. Microorganisms are distributed throughout the entire product during the various processing steps. Product temperature is the major factor that affects microbial growth during the interval between manufacture and consumption. Past research (*5*) has revealed that the microbial quality or shelf-life of ground beef can be improved if the temperature remains below -1 C. It is essential to control microbial growth to preserve a natural and fresh flavor with an ultimate increase in stability through reduced deterioration in appearance and flavor.

Ground beef is frequently purchased as a fresh product, and it is then frozen and later thawed and cooked. Since limited information related to characteristics of thawed meat is available, this research was conducted to evaluate the effect of thaw time and temperature of the thawing environment on the appearance, taste and microbial population of frozen ground beef.

MATERIALS AND METHODS

Sixty-six fresh ground beef samples, each of which weighed approximately 300 g, were randomly selected from the meat cases of two retail food stores in east Texas. Purchases from these outlets were made on Monday, Wednesday and Friday to acquire a representative cross-section of ground beef manufactured from beef trimmings and merchandised during the week. Immediately after purchasing, six of the samples were evaluated by four raters who scored each sample for color and overall appearance by use of 8-point rating scales. Rating scale nomenclature was as follows: color (8 = very bright red; 1 = gray or green discoloration) and overall appearance (8 = extremely desirable; 1 = extremely undesirable).

After initial evaluation, the packaged ground beef samples were frozen and stored at -25 C. Following two weeks of storage, one-half of the packages were thawed at 5 C for 4, 8, 24, 48 and 72 h, whereas the other samples were subjected to a 25-C environment for the same periods. At the end of each thaw period, samples were evaluated for the same characteristics as before freezing.

In addition to evaluation for appearance traits, ground beef from each treatment was evaluated by four sensory panel members for flavor, odor and overall acceptability after 4-8 weeks of storage at -25 C. The following rating scales were used for flavor, odor and overall taste: (8 = extremely desirable, 1 = extremely undesirable). Samples were thawed at 5 C for 3-4 h and broiled at 170 C to an internal temperature of 70 C.

The microbial load of each ground beef sample was determined by blending 25 g with 225 ml of 0.1% peptone broth (as a diluent) in a sterile blender jar. After serial dilutions were made, the samples were introduced into various selective broths and then onto selective plating media as described in Table 1 for determination of coliforms, total plate count, *Escherichia coli*, staphylococci, streptococci, *Pseudomonas*, *Salmonella*, and lactobacilli. Procedures reported by Warseck et al. (*18*) and Ray and Speck (*13*) for repair and enumeration of injured coliforms in frozen foods were utilized as guidelines. Further identification of microorganisms was made through procedures outlined in *Bergey's Manual of Determinative Bacteriology* (*1*) and the U.S.D.A. *Microbiology Laboratory Guidebook* (*17*). Other microorganisms not previously mentioned were identified through use of techniques such as the oxidase test, gram staining, morphology and API strips.

The data, as logarithms of the mean microbial counts, and all subjective ratings for appearance and taste were treated by factorial analysis of variance (*14*). Mean separation analysis by the Duncan's Multiple Range Test (*4*) was done on the levels of those factors that

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produced significant main effects. Multiple regression analysis and correlation coefficients were provided to determine a relationship among certain variables. Statistical procedures available on the BMDP statistical package, as implemented on an IBM 360/50, were used for all statistical evaluations (*3*). Microbial counts were coded [(MC + 1) × 10] to preclude analysis of data with zero values and logarithms that were less than one. In tables where actual bacterial numbers were presented, the 1.0 was removed from each value.

RESULTS AND DISCUSSION

Since past research has not quantified how different thaw periods and temperatures affect the appearance, taste and microbiology of ground beef, these data will provide observed trends from treatments previously discussed.

Appearance traits

Evaluation of the ground beef samples for color (Table 2) revealed that those thawed at 25 C sustained more ($P < .05$) color degradation after 24 h than those stored for less time. Ground beef thawed for more than 24 h at 25 C had lower ($P < .05$) color scores than that stored for less time. However, thaw time did not significantly affect color degradation for those periods of less than 24 h at 5 or 25 C. Samples exposed for 24 h at 5 C did not differ ($P < .05$) from each other in color from any other periods at that temperature. This observation suggests that thawing for up to 48 h at refrigerated temperature will still give an acceptable appearance. This trend is evident because color did not differ significantly from samples with a shorter thaw time, even though scores for shorter periods were numerically higher. Less variation in color scores at 5 C was attributed to lower initial scores due to some loss of color through display and freezing. In addition to more ($P = .05$) color degradation at 24 h than for shorter thaw periods at 25 C, those samples held for 72 h had more ($P < .05$) color deterioration than ground beef thawed for 24 h. Ground beef samples held at 25 C for 48 and 72 h were rated very low in color and were not significantly different. Color of ground beef samples held for 4 and 8 h did not differ significantly from each other due to thaw temperature, whereas samples held for 24 h and longer at 25 C sustained more ($P < .05$) color degradation than counterparts at 5 C. These data suggest that ground beef should be thawed at 5 C to reduce color degradation, especially if the product is held beyond 8 h.

Effects of thaw time and temperature on overall appearance of ground beef are presented in Table 3.

TABLE 2. *Color scores[ab] of ground beef with different thaw periods and temperatures.*

Thaw time (h)	Thaw temperatures: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	5.50[c]	1.00	5.17[c]	1.13
8	5.50[c]	1.01	4.85[c]	1.32
24	5.25[cd]	1.00	2.85[d]	0.58
48	4.88[cd]	0.95	1.92[de]	0.75
72	4.17[d]	0.90	1.54[e]	0.70

[a]Means are based on an 8-point scale (8 = very bright red; 1 = gray or green discoloration).
[b]Means on the same row that are underlined are not different ($P > .05$).
[cde]Means in the same column bearing a common superscript letter are not different ($P > .05$).

TABLE 3. *Overall appearance scores[a] of ground beef with different thaw periods and temperatures.*

Thaw time (h)	Thaw temperatures: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	5.62[b]	0.79	5.33[b]	0.34
8	5.87[b]	0.56	5.18[b]	0.87
24	5.29[b]	0.53	2.85[c]	0.49
48	5.04[bc]	0.45	1.67[d]	0.49
72	4.25[c]	0.92	1.38[d]	0.34

[a]Means are based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcd]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

Thaw time and temperature had an effect on overall appearance similar to that of color, since no significant differences existed among all samples thawed at 5 C for 48 h or less. However, ground beef thawed at 5 C for 72 h was less ($P < .05$) desirable in appearance than that held for 24 h or shorter periods. Ground beef thawed for 4 and 8 h at 25 C was more ($P < .05$) desirable in appearance than those samples held for 24 h, whereas those thawed for 24 h were superior ($P < .05$) in appearance to samples held for 48 to 72 h. Differences in appearance between ground beef thawed 48 and 72 h were not significant and both were not acceptable in appearance and were rated very low. Thaw temperature did not significantly affect overall appearance during the first 8 h of storage; however, beyond this period those stored at 5 C were more ($P < .05$) desirable in appearance than those held at 25 C. This trend suggests further need to thaw at refrigerated temperature, especially when time exceeds 8 h.

Microbial load

When ground beef was thawed at 5 C, thaw time

TABLE 1. *Media and incubation conditions for microbial determinations.*

Microorganisms	Selective broth	Media	Incubation: Time (h)	Incubation: Temperature (C)
Aerobes (total plate count)	—	Standard Methods agar	48	25
Coliforms	Trypticase Soy broth	Violet Red Bile agar	48	25
Escherichia coli	Brilliant Green Lactose Bile	Levin Eosin Methylene Blue agar	24	37
Lactobacilli	Trypticase Soy broth	Lactobacillus Selection agar	48	25
Pseudomonas	Trypticase Soy broth	Pseudomonas Isolation agar	48	25
Salmonella	Selenite Cystine broth	Xylose Lysine Desoxycholate agar	24 & 48	37
Staphylococci	Trypticase Soy broth (10% salt)	Mannitol Salt agar	48	37
Streptococci	Azide Dextrose broth	K F Streptococcal agar	48	37

through 72 h did not affect ($P < .05$) the total plate count (Table 4). At 25 C, microbial proliferation did not differ significantly between 4 and 8 h. However, ground beef held for 24 h at 25 C had higher ($P < .05$) counts than samples thawed for less time. Samples removed from frozen storage and stored for 48 and 72 h at 25 C did not differ significantly from each other in total plate count, but had higher ($P < .05$) counts than those stored for 24 h. These data verify need to thaw ground beef at a refrigerated temperature or for a maximum of 8 h if left outside a refrigerated environment. Higher counts from samples thawed beyond 8 h at 25 C paralleled degradation of color and overall appearance. This observation was attributed to action of the greater number of microorganisms resulting from increasing thaw time and temperature. Although temperature had no significant effect on microbial growth through 8 h, samples held longer in the 25-C environment had more ($P < .05$) microorganisms than those kept at 5 C.

Taste appeal

Information related to effect of different thaw times and temperatures is lacking. This study (Tables 5, 6, and 7) illustrated how thaw time and temperature affected taste attributes of ground beef. These data indicate that flavor traits were not significantly different at various thaw times at 5 C, except those samples which were held for 4 h were more ($P < .05$) desirable in flavor and overall taste than those held for 48 and 72 h. Odor scores for samples thawed for 4 h at 5 C were higher ($P < .05$) than for those held for 72 h, but samples from other storage periods at 5 C were not significantly different. At 25-C storage, none of the taste characteristics were significantly different between 4 and 8 h but were more ($P < .05$) desirable than those thawed for 24 h. Samples held for 48 and 72 h were not significantly different from each other but were less ($P < .05$) desirable than counterparts maintained at 25 C. These observations imply that to maintain acceptable taste characteristics, ground beef should not be thawed more than 24 h at 5 C or 8 h at 25 C. Although data were not significant ($P > .05$), ground beef thawed for 4 h had consistently higher scores than the samples evaluated at 8 h. This observation suggests that optimal acceptability is attained with a shorter thaw time.

TABLE 4. *The effect of thaw time and temperature on total plate count (log_{10} per g) of ground beef.*

Thaw time (h)	Thaw temperatures: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	5.12[a]	0.35	5.36[a]	0.95
8	5.84[a]	0.96	5.65[a]	0.99
24	5.40[a]	0.59	7.57[b]	0.56
48	5.07[a]	0.39	8.55[c]	0.20
72	5.78[a]	0.49	8.54[c]	0.63

[abc]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

TABLE 5. *The effect of thaw time and temperature on odor scores[a] on ground beef.*

Thaw time (h)	Thaw temperatures: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	6.13[b]	0.49	6.25[b]	0.47
8	5.92[bc]	0.56	5.89[b]	1.39
24	5.46[bc]	0.93	4.60[c]	1.39
48	5.50[bc]	0.69	2.04[d]	0.71
72	5.21[c]	0.66	1.58[d]	0.20

[a]Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcd]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

TABLE 6. *The effect of thaw time and temperature on flavor scores[a] of ground beef.*

Thaw time (h)	Thaw temperatures: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	6.13[b]	0.80	6.21[b]	0.60
8	5.83[bc]	0.56	5.79[b]	1.56
24	5.45[bc]	0.97	4.00[c]	1.53
48	5.08[c]	0.54	1.71[d]	0.62
72	5.08[c]	0.54	1.29[d]	0.29

[a]Means are based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcd]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

TABLE 7. *The effect of thaw time and temperature on overall taste scores[a] of ground beef.*

Thaw time (h)	Thaw temperature: 5 C $\bar{x}$	5 C SD	25 C $\bar{x}$	25 C SD
4	6.17[b]	0.65	6.33[b]	0.44
8	5.83[bc]	0.56	5.86[b]	1.48
24	5.50[bc]	0.88	4.05[c]	1.59
48	5.00[c]	0.63	1.67[d]	0.58
72	5.00[c]	0.63	1.17[d]	0.26

[a]Means are based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcd]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

Statistical relationships

Data generated from this study were used to establish a relationship between various measurements. The relationships were determined by conducting multiple regression analyses to predict microbial load and flavor. Coefficients of determination (R^2) from the multiple regressions which are given in Table 8 were all significant ($P < .05$) except for those values for microbial load at 5 C.

A prediction equation was developed to predict microbial load and flavor for each thaw temperature according to the following model:

$$\hat{Y} = a + b_1x_i + b_2x_i + b_3x_i + b_4x_i + b_5x_i$$

where for microbial load:

$\hat{Y}$ = microbial load
a = intercept coefficient
b_1 = overall appearance coefficient
b_2 = color coefficient
b_3 = odor coefficient
b_4 = overall taste coefficient
b_5 = flavor coefficient

and for flavor:

$\hat{Y}$ = flavor
a = intercept coefficient
b_1 = overall acceptability coefficient
b_2 = color coefficient
b_3 = odor coefficient
b_4 = overall taste coefficient

It may be seen that the coefficients of determination (R^2) noted in Table 8 improve with the higher thaw temperature. This trend is especially apparent for microbial load. The greater variation in measurements at 25 C creates a possibility for larger coefficients of determination. Multiple regression analysis suggests that all variables measured at both thaw temperatures are highly indicative of anticipated flavor. Although variables measured at 25 C reflect a relationship to anticipated microbial load, a small relationship exists between these same variables measured at 5 C.

Correlations among variables are given in Table 9. A significant ($P < .05$) correlation existed between flavor and all taste and appearance traits at both thaw temperatures. There was a significant ($P < .05$) negative correlation between microbial load and all varialbes at 25 C, but those coefficients at 5 C were not significant. This difference was attributed to less variation of values obtained at 5 C. Interpretation of these data suggests that flavor was closely associated with all appearance and taste attributes and that an increase in microbial load was responsible for degradation of taste and appearance traits.

Taxonomy

Microbial taxonomy revealed that aerobic bacteria were most abundant among all thaw periods at both temperatures. No *Salmonella* or *Escherichia coli* were recovered from any treatment. Other workers (*2,16*) have reported that these microbes are infrequently found and are present in small quantities. As indicated in Table 10, lactobacilli and coliforms were found among the various treatments in small proportions. However, as thaw time exceeded 24 h, the percentage of lactobacilli increased among samples held at 5 C and coliform counts increased among samples thawed at 25 C. These data illustrate that as thaw time increased, lactobacilli that were initially present were capable of establishing themselves enough at 5 C with increased storage time to cause a reduction in flavor desirability. Likewise, extended storage at 25 C was responsible for a higher proportion of coliforms in the microbial population, thus preventing a less wholesome foodstuff with extensive flavor degradation.

TABLE 8. *Coefficients of determination (R^2) between microbial load and flavor and other variables.*[a]

Variables	Microbial load		Flavor	
	5 C	25 C	5 C	25 C
Overall appearance	—	0.680	0.937	0.993
Color	0.057	0.718	0.939	0.993
Odor	0.024	0.707	0.935	0.993
Flavor	0.034	0.716	—	—
Overall taste	0.002	0.705	0.936	0.994

[a]All R^2 values are significant ($P < .05$) except for those of microbial load in the 5 C column.

TABLE 9. *Correlation of regressions among microbial load and flavor with other variables.*[a]

Variables	Microbial load		Flavor	
	5 C	25 C	5 C	25 C
Overall appearance	—	−0.831	0.325	0.905
Color	−0.299	−0.715	0.229	0.850
Odor	−0.139	−0.745	0.873	0.983
Flavor	−0.063	−0.744	1.000	1.000
Overall taste	−0.076	−0.757	0.968	0.996

[a]All correlations are significant ($P < .05$) except for those of microbial load in the 5 C column.

Random selection of isolates was accomplished by choosing sample bacterial colonies from each treatment with variation in morphology and pigmentation. Classification indicated that the most abundant among the isolates were micrococci, *Pseudomonas*, streptococci, staphylococci and *Flavobacterium*. Earlier research (*7,10,11*) has revealed that a wide variety of microorganisms, including gram-negative and gram-positive microflora, are responsible for deterioration of appearance and taste attributes of aerobically stored meat. The microbial flora isolated from frozen meats has been previously identified (*12*). A larger proportion of micrococci than *Pseudomonas* was attributed to sensitivity of the latter to freezing and storage (*9*).

Microorganisms responsible for major public health hazards such as *Salmonella, Bacillus, Escherichia coli* or *Staphylococcus aureus* were not isolated during the taxonomy process. A survey conducted by Surkiewicz et al. (*16*) revealed that a higher percentage of raw ground beef patties did not have enough of these microbes to present a public health concern, whereas Cremer and Chipley (*2*) concluded that potential for public hazards exists if precooked patties are mishandled. The major hazard associated with extended thaw time, especially at 25 C, was deterioration of appearance and flavor of the meat. Microorganisms that were well established in the population during freezing and when thaw time started

TABLE 10. *Microbial taxonomy of ground beef with different thaw periods and temperatures.*

Microflora	4 h		8 h		24 h		48 h		72 h	
	5 C	25 C	5 C	25 C	5 C	25 C	5 C	25 C	5 C	25 C
Total plate count microbes	98.54	99.55	99.95	99.33	99.67	91.60	94.07	93.18	82.33	85.22
Lactobacillus sp.	0.14	0.03	0.02	0.04	0.29	0.27	4.72	0.09	16.91	2.81
Coliforms	1.32	0.42	0.03	0.63	0.04	8.13	1.21	6.73	0.76	11.97

appeared to create enough competition to prevent much of a population shift until increased growth of lactobacilli and coliforms occurred at 5 and 25-C storage, respectively, after 48 h of thaw time.

CONCLUSIONS

These data support the following conclusions: (*a*) Ground beef thawed beyond 48 h at 5 C was less ($P < .05$) desirable in apperance traits. (*b*) Ground beef thawed beyond 24 h at 5 C was less ($P < .05$) desirable in taste attributes, thus suggesting that thaw time at refrigerated temperature should not exceed 24 h. (*c*) The thaw time beyond 8 h at 25 C was responsible for more ($P < .05$) degradation in appearance and taste characteristics than a shorter time, therefore demonstrating need for thawing ground beef for 8 h or less outside refrigerated temperature range. (*d*) Storage of frozen ground beef beyond 8 h at 25 C was responsible for increased ($P < .05$) microbial load; consequently verifying that ground beef should be thawed 8 h or less at ambient temperature or thawed at refrigerated temperature if thaw time exceeds 8 h. (*e*) Variables measured subjectively, i.e., color, overall appearance, odor and overall taste, were accurate predictors of flavor at both thaw temperatures and of microbial load when thawed at 25 C. These traits did not accurately predict microbial load for 5 C thaw temperature since microbial counts were not significantly different among treatments at this temperature. (*f*) All traits were highly ($P < .01$) correlated for those samples thawed at 25 C but most traits were not as highly correlated among samples thawed at 5 C, but at 5 C odor and overall acceptability of taste were highly correlated with flavor. (*g*) Micrococci, streptococci, staphylococci and *Flavobacterium* were the most abundant. As thaw time at 5 C increased, lactobacilli became more established, whereas samples held at 25 C contained more coliforms.

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Appearance and Microbial Quality of Thawed Retail Cuts of Beef, Pork and Lamb[1]

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ABSTRACT

Retail cuts of beef, pork and lamb were fabricated, packaged, evaluated for appearance traits, swabbed, and subsequently frozen. After storage for 7 days, one-half of the cuts were thawed at 4 C. The other cuts were thawed at 25 C. At 0, 4, 8, 24 and 48 h, the thawed retail cuts of meat were rated for appearance and swabbed. Taxonomy was conducted before freezing and after each thaw period. Evaluation revealed that differences ($P < .05$) in appearance for beef and pork existed between those samples thawed for 24 h and 0 h. Beef and pork cuts thawed at 25 C were less ($P < .05$) desirable in appearance after 24 h than for 8 h. Cuts thawed at 4 C for 48 h were less desirable than for 24 h. A higher thaw temperature increased ($P < .05$) microbial growth at 24 h on beef; but this trend was not observed among cuts of lamb and pork. Thaw time beyond 24 h increased ($P < .05$) the microbial load of only lamb and pork. Microorganisms most prevalent among samples were *Pseudomonas,* micrococci and staphylococci. These data suggest that retail cuts of meat should be thawed at refrigerated temperature, especially if thaw time exceeds 8 h.

Appearance and bacteriological condition of meat is of special interest to the food industry and the consumer. It has been known for some time that microorganisms cause meat color and flavor degradation (*2,15*). Past studies have enumerated microbial flora of fresh ground beef and other products (*5,7,8,15,19,26,27,28,31*). Other research (*13,23*) has determined the effect of freezing on the microbial load of meat.

A trend has existed among consumers (*32*) to purchase fresh retail cuts of meat from a grocery store, place the meat in a refrigerator or freezer for freezing and later thaw the meat before cookery. Previous research (*22*) on microbial quality of retail cuts of meat has determined that freezing extends the lag phase of microbial proliferation. Additional work (*23*) suggested that freezing can be responsible for some decrease in microbial load due to metabolic injury of bacterial cells.

According to results of previous investigations (*24,34*), bacterial genera are more critical to spoilage than is total microbial load. These observations are supported by results which indicate that certain genera cause discoloration, undesirable flavor and food poisoning at lower ($P < .05$) levels of contamination than do other microorganisms. Other research (*9*) has revealed that various food poisoning microorganisms are unable to compete effectively with the natural flora of raw ground beef over a wide range of refrigerated temperatures. These workers' results and the fact that a cooking step is involved (*12*) explain why this product is rarely involved as a vehicle in food poisoning by microorganisms.

Investigations have shown that fresh meats at the retail level have high bacterial counts (*4,14,16,17,25*). A survey conducted (*31*) of raw beef patties produced at federally inspected plants revealed that bacteriological quality of the product was a measure of the cumulative increase of bacterial growth on meat from the time of slaughter through manufacture. Although it is difficult to demonstrate a real or potential health hazard from contamination or time-temperature abuse, microbial load may be related indirectly to a health hazard (*6*). Thus additional study of thaw time and temperature seems appropriate to determine what constitutes time-temperature abuse and to provide additional information of interest to the industry and the consumer.

Although results of storage of fresh and frozen retail cuts of meat for various periods have been documented, very little is known about resultant effects of various thaw periods at different temperatures on the appearance and microbial load of such meat. Therefore, this investigation was designed to evaluate the role of two thaw temperatures and five thaw periods on the stability of beef, pork and lamb cuts.

MATERIALS AND METHODS

Beef loin steaks, pork loin chops and lamb chops were fabricated from wholesale loins, aerobically packaged with polyvinyl chloride film as retail cuts of meat and displayed in a fresh meat case. The beef steaks were cut from loins that were previously cut and vacuum-packaged by a beef fabricator. The pork loins were fabricated by a pork slaughtering firm, wrapped in parchment, shipped to a retail store and then cut into chops, whereas the lamb loins were cut from lamb carcasses by a lamb slaughtering firm and shipped unwrapped to the retail store for retail cutting and packaging. Retail cuts from beef and pork were fabricated, packaged and displayed in the same store in east Texas, but the lamb chops were produced in another store in the same geographic area. Beef and pork cuts were packaged the day of purchase, but the lamb chops had been packaged and displayed for two days before being acquired. These retail cuts of meat were randomly selected from the display case and purchased. Enough samples were secured to provide five replications per treatment. The samples were

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then taken directly to the laboratory to be subjectively rated by three skilled evaluators for color and overall appearance by use of 8-point rating scales. Rating scale nomenclature was: color (8 = extremely desirable; 1 = extremely undesirable).

Microbial load was determined by use of the swab technique and subsequently plating with Standard Methods agar. Serial dilutions were made and the samples were introduced into various selective broths. The samples were subsequently transferred onto selective plating media as described in Table 1 for determination of total plate count, coliforms, staphylococci, streptococci and *Pseudomonas*. Procedures reported by Warseck et al. (*35*) and Ray and Speck (*20*) for repair and enumeration of injured coliforms in frozen foods were utilized as a guideline. Further identification of microorganisms was conducted according to other procedures (*1,33*) available to determine population mixture of the microbial flora. Other microorganisms not previously mentioned were identified through use of techniques such as the oxidase test, gram staining, morphology and API strips.

After initial determinations for appearance and microbial flora, the samples were frozen and stored at -25 C. Following 2 weeks of storage, one-half of the cuts were thawed at 4 C for 0, 4, 8, 24 and 48 h; whereas, the other samples were subjected to a 25-C environment for the same periods. At the end of each thaw period, all of the samples were subjected to the same evaluations conducted before freezing.

The data, as logarithms of the mean counts, and all subjective ratings for appearance were treated by factorial analysis of variance (*29*). Mean separation analysis by orthogonal decomposition and contrast analysis by the student's t-test (*30*) was performed on the levels of those factors that produced significant main effects. Multiple regression analysis and correlation coefficients were provided to establish a relationship between certain variables. Statistical procedures available on the BMDP statistical package as implemented on an IBM 360/50 were used for all statistical evaluations (*3*). Microbial counts were coded $[(MC + 1) \times 10]$ to preclude analysis of data with zero values and logarithms that were less than one. In tables where actual bacterial numbers were presented, the 1.0 was removed from each value.

RESULTS AND DISCUSSION

Appearance traits

Evaluation of beef steaks for color (Table 2) revealed that those thawed at 25 C sustained more ($P < .05$) color degradation after 4 h than did those with a shorter thaw time. For each thaw period beyond 4 h, steaks were less ($P < .05$) desirable in color than for the prior period. The same trend existed for those cuts thawed at 4 C, except that significant differences did not occur until after 8 h. Samples which were thawed for 8 h or longer sustained more ($P < .05$) color degradation at 25 C than 4 C. Steaks thawed for 24 h at 4 C were still acceptable in color, whereas those held at 25 C were only acceptable up to 8 h. These data suggest that 24 h was an acceptable thaw period under refrigeration (4 C). At 25-C storage, 8 h was the maximum storage time.

TABLE 2. *The effect of thaw temperature and time on color scores for beef loin steaks.*[a]

Thaw time (h)	Thaw temperature 4 C		25 C	
	$\bar{X}$	(SD)	$\bar{X}$	(SD)
Pre-freeze	7.7[b]	.30	7.7[b]	.30
0	6.3[c]	.37	6.5[c]	.30
4	6.1[c]	.15	6.0[c]	.00
8	5.9[c]	.18	5.2[d]	.18
24	5.6[d]	.44	3.1[e]	.28
48	4.5[e]	.45	1.0[f]	.00

[a]Means are based on an 8-point scale (8 = very bright red; 1 = gray or green discoloration).
[bcdef]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

Table 3 suggests that color degradation of pork loin chops had a pattern similar to that of beef cuts. One noted difference was that pork color was more stable since no significant differences between thaw periods existed until the pork loin chops were thawed for 24 h at 4 C and 4 h at 25 C. High initial scores for pork loin chops indicated that these cuts were very fresh at the time of purchase. Slightly higher ratings for pork chops than for beef steaks during the thaw periods suggested that superior initial condition and greater color stability of the pork samples contributed to less color degradation. Since the pork samples were lighter pigmented than beef, initial changes in color of the pork was less obvious. Pork samples were more desirable ($P < .05$) in color after 8 h at 25 C than beef samples thawed for 4 h at 25 C, further suggesting that a fresher condition before freezing contributed to color stability of pork during thawing. Small standard deviations of color scores for pork chops suggested that skilled raters perceived little

TABLE 3. *The effect of thaw temperature and time on color scores for pork loin chops.*[a]

Thaw time (h)	Thaw temperature 4 C		25 C	
	$\bar{X}$	(SD)	$\bar{X}$	(SD)
Pre-freeze	8.0[b]	.00	8.0[b]	.00
0	7.4[b]	.15	7.7[b]	.15
4	7.4[b]	.28	6.5[c]	.19
8	7.0[b]	.00	6.3[c]	.15
24	6.3[c]	.01	5.1[d]	.30
48	5.9[d]	.15	2.5[e]	.38

[a]Means are based on an 8-point scale (8 = very bright red; 1 = gray or green discoloration).
[bcde]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

TABLE 1. *Media and incubation conditions for microbial determinations.*

Microflora	Selective Broth	Media	Incubation Time	Incubation Temperature
Aerobes (total plate count)	—	Standard Methods Agar	48 h	25 C
Coliforms	Trypticase Soy	Violet Red Bile Agar	48 h	25 C
Escherichia coli	Brilliant Green Lactose Bile	Levine Eosin Methylene Blue Agar	24 h	37 C
Pseudomonas	Trypticase Soy	*Pseudomonas* Isolation Agar	48 h	25 C
Staphylococci	Trypticase Soy (10% salt)	Mannitol Salt Agar & Baird-Parker Agar	48 h	37 C
Streptococci	Azide dextrose	KF Streptococcal Agar	48 h	37 C

variation between replications among various treatments.

Color scores for lamb loin chops (Table 4) were less variable during thawing than scores for beef and pork cuts. This condition apparently was due to chops that reflected more deterioration at the time of purchase, as evidenced by lower initial scores. Greater initial degradation of color and increased stability of myoglobin appeared to reduce variation from freezing through thawing at various intervals. Although lamb loin chops did not deteriorate significantly in color throughout all thaw periods at 4 C, those samples thawed for 8 h or longer at 25 C sustained more ($P < .05$) color degradation than those chops which were thawed for 4 h or less. Small standard deviations were attributed to more homogeneity of lamb chops due to fewer samples for selection from the retail case. The limited number of samples was obviously taken from a limited number of lamb loins.

Table 5 presents overall appearance scores for beef loin steaks subjected to the same thaw time and temperature treatments as previously described in Table 2. Data for overall appearance indicate a trend similar to color evaluations because of similar values and thaw periods where significant differences were found. Standard deviations were also similar and a high correlation between color and overall appearance scores suggests why results were similar. Overall appearance scores of pork and lamb cuts are not presented since they reflected a relationship to color scores similar to that of the beef samples.

TABLE 4. *The effect of thaw temperature and time on color scores for lamb loin chops.*[a]

Thaw time (h)	Thaw temperature 4 C		25 C	
	$(\bar{X})$	(SD)	$(\bar{X})$	(SD)
Pre-freeze	5.0[b]	.00	5.0[b]	.00
0	5.0[b]	.00	5.0	.00
4	5.0[b]	.00	4.5[b]	.32
8	4.9[b]	.22	3.9[c]	.22
24	5.0[b]	.00	3.9[c]	.22
48	4.7[b]	.27	3.5[c]	.32

[a]Means are based on an 8-point scale (8 = very bright red; 1 = gray or green discoloration).
[bc]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

TABLE 5. *The effect of thaw temperature and time on overall appearance scores for beef loin steaks.*[a]

Thaw time (1)	Thaw temperature 4 C		25 C	
	$(\bar{X})$	(SD)	$(\bar{X})$	(SD)
Pre-freeze	7.7[b]	.30	7.7[b]	.30
0	6.9[c]	.15	6.9[c]	.15
4	6.9[c]	.18	6.9[c]	.15
8	6.3[c]	.37	5.5[d]	.19
24	5.8[d]	.18	3.2[e]	.18
48	5.0[e]	.47	1.1[f]	.18

[a]Means are based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[bcdef]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

Microbial load

The effect of thaw time and temperature on total plate count for beef loin steaks is illustrated in Table 6. At 4-C storage, microbial load did not significantly change as thaw time increased. This trend suggested that thawing at this temperature is advantageous since microbial proliferation is retarded. Although microbial counts did not significantly differ from the time of sampling through 8 h of thaw time at 25 C, those samples thawed for 24 and 48 h at 25 C sustained more ($P < .05$) proliferation of microbial flora than steaks thawed for 8 h or less. Higher counts after 8 h of storage at 25 C suggested the importance of storage at a refrigerated temperature if thaw time exceeds 8 h.

Microbial load for pork and lamb cuts is not included in tabular form since those results closely paralleled counts for beef steaks. One difference was that pork chops stored at 4 C for 48 h had significantly higher counts than those thawed for less time. This trend suggested that microbial proliferation increased at a faster rate for pork than beef since at this thaw time pork chops contained a higher percentage of cold-tolerant *Pseudomonas* than beef (Table 7). The microbial population on lamb loin chops exhibited a growth pattern similar to that on pork except that those samples stored for 24 h at 4 C had significantly more growth of microorganisms than did those thawed less than 24 h. This trend also existed among lamb chops stored between 24 and 48 h at 4 C. More rapid microbial growth on lamb chops thawed at 4 C was attributed to a higher percentage of cold tolerant *Pseudomonas* with less thaw time than for pork (Table 7) and because of lamb chops that were not as fresh as the other species at the time of sampling.

TABLE 6. *The effect of thaw temperature and time on total plate count (log_{10} per 6.46 cm^2) for beef loin steaks.*

Thaw time (h)	Thaw temperature 4 C		25 C	
	$(\bar{X})$	(SD)	$(\bar{X})$	(SD)
Pre-freeze	5.0[a]	.84	5.0[a]	.52
0	3.8[a]	.45	4.4[a]	.41
4	4.2[a]	.07	4.5[a]	.14
8	4.3[a]	.11	4.3[a]	.53
24	5.2[a]	1.67	8.5[b]	1.22
48	4.3[a]	.45	9.0[b]	.30

[ab]Means in the same rows and columns bearing a common superscript letter are not different ($P > .05$).

Taxonomy

Microorganisms are listed according to predominant species and thaw time in Table 7. The most abundant microbe on beef steaks at the time of sampling was *Microbacterium thermosphactum.* It was discovered that the steaks acquired for this study had been cut from vacuum-packaged beef short loins. This microorganism has been found to be prominent among vacuum-packaged cuts of beef by other researchers (*10,11,17*). The microorganism that most successfully withstood freezing and frozen storage was *Micrococcus.* Other

workers (*18,21,23*) have indicated that freezing may cause metabolic injury to bacterial cells. These authors have indicated that some recovery time is needed during thawing and that *Micrococcus* is one of the most abundant microbes immediately after frozen storage. Although *Pseudomonas* increased during each thaw period, *Micrococcus* remained the most abundant on beef steaks throughout all storage periods.

Pork samples that had been aerobically packaged since fabrication were found to have predominantly *Pseudomonas* before freezing. Research before this study (*15,16,17,27*) has revealed that *Pseudomonas* is the most abundant among aerobically packaged red meats that have not been cured or frozen. For pork samples, staphylococci were less resistant to freezing and frozen storage than other microorganisms. Other research (*18*) has indicated that this microorganism is one of the more predominant among frozen meat at 35-C incubation. As thaw time increased, a microbial population shift appeared to occur since *Pseudomonas* became more abundant and the amount of other microbes decreased.

The most abundant microorganism among aerobically packaged lamb chops before freezing was *Pseudomonas*. As with beef samples, *Micrococcus* was the most resistant to freezing and frozen storage. After the lamb chops were subjected to thawing, *Pseudomonas* became the most abundant microorganism. A population shift of microbial flora on lamb chops similar to that of the pork samples appeared to occur, except that it transpired earlier during the thaw process. A more rapid shift to a population dominated by *Pseudomonas* among thawed lamb chops than for beef and pork cuts appeared to be caused by a higher percentage of this microorganism among lamb samples before freezing and frozen storage. Other microorganisms (i.e., streptococci and coliforms) were identified, but not consistently.

Statistical relationships

Table 8 contains a correlation matrix which illustrates the relationship between color scores, overall appearance scores and microbial load. As previously noted, a close positive relationship existed between color scores and overall appearance scores. Therefore, those samples that sustained less color degradation were also more desirable in appearance. Although color scores and appearance scores were not as highly correlated with microbial load as with each other, these negative correlations revealed that higher microbial counts contributed significantly to lower color scores and overall appearance scores. Appearance scores were more closely related to microbial load than were color scores.

TABLE 8. *Correlation matrix for color, overall appearance and microbial load of retail cuts.*

	Color	Appearance	Microbial load
Color	1.00	—	—
Appearance	0.80	1.00	—
Microbial load	-0.36	-0.58	1.00

Multiple regression analysis was performed on microbial load, color and overall appearance to develop the following highly significant ($P < .001$) prediction equation:

$$\hat{Y} = a + b_1x_1 + b_2x_1$$

where Y = microbial load
a = intercept coefficient
b_1 = color coefficient
b_2 = overall appearance coefficient

Analyses revealed the following results:

$a = 10.483$
$b_1 = -.389$
$b_2 = -1.067$
multiple $R^2 = .3694$

These data suggested that color and overall appearance scores were related to 36.94% of the variance in microbial load.

CONCLUSIONS

These data support the following conclusions: (*a*) Elevated thaw temperature (25 C) and increased thaw time were related to color degradation and reduced overall appearance of frozen steaks and chops. (*b*) Elevated thaw temperature and increased thaw time were related to additional microbial proliferation. (*c*) *Pseudomonas* predominated in the microbial population of meat that had been aerobically packaged as primal and retail cuts before freezing. (*d*) A fluctuation of microbial population mixture was obtained after freezing and frozen storage which was followed by another population change as thaw time increased. (*e*) Color scores and overall appearance scores accounted for approximately 37% of the variance in microbial load. (*f*) Frozen retail cuts of meat should be thawed at refrigerated temperature (4 C) for less than 24 h to maintain optimal appearance and minimal microbial contamination.

ACKNOWLEDGMENTS

The authors extend acknowledgment to Wilson Howell and David Murphree for laboratory contribution to this work.

TABLE 7. *Predominant microflora according to species and thaw time at 4 C and 25 C.*

Time (h)	Species		
	Beef	Pork	Lamb
Pre-freeze	*Microbacterium thermosphactum*	*Pseudomonas* sp.	*Pseudomonas* sp.
0	*Micrococcus* sp.	*Staphylococcus* sp.	*Micrococcus* sp.
4	*Micrococcus* sp.	*Staphylococcus* sp.	*Pseudomonas* sp.
8	*Micrococcus* sp.	*Staphylococcus* sp.	*Pseudomonas* sp.
24	*Micrococcus* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.
48	*Micrococcus* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.

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psychrotrophic methods of raw and low-count pasteurized milk products. More rapid methods are needed which correlate with keeping quality information. (Dr. Robert T. Marshall, Department of Food Science and Nutrition, 203 Eckles Hall, University of Missouri, Columbia, MO 65201; phone: 314-882-7355).

5. *Standard Samples for Instrument Calibration.* Procedures need to be refined for preparing stable samples for calibration of the electronic instrumentation used in composition and somatic cell analysis of milk. (Mr. Roy Ginn, Dairy Quality Institute, 2353 N. Rice St., St. Paul, MN 55113; phone: 612-484-7269).

6. *Analytical Methods for Sanitizing Agents.* Procedures for analysis of quaternary ammonium, iodophor, and other sanitizing agents need development. (Dr. John C. Bruhn, Extension Food Technologist, Department of Food Science and Technology, 101 Cruess Hall, University of California, Davis, CA 95616; phone: 916-752-2192.

7. *Surface or Spread Plate Method.* A collaborative study comparing a surface plate and Standard Plate Count methods is suggested. Incorporation of an .001-ml loop or .01-ml cylinder sample-diluent delivery system might be considered. (Dr. John C. Bruhn, Extension Food Technologist, Department of Food Science and Technology, 101 Cruess Hall, University of California, Davis, CA 95616; phone: 916-752-2192).

8. *Total Solids Analyses.* Microwave oven techniques have allowed rapid estimation of solids in various dairy foods. Modifications of time-temperature relationships need to be established for various dairy food products. Similarly, infra-red instruments are being evaluated for anal-

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Journal of Food Protection Vol. 43, No. 3, Page 190 (March, 1980)

A Research Note

Effect of Electrical Stimulation on Meat Spoilage Floras

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ABSTRACT

Sheep carcasses were electrically stimulated after removal of one leg. There was no difference between the growth of spoilage bacteria on stimulated and non-stimulated legs, or in minces prepared from either type of leg, with either the natural or an inoculated flora.

A recent paper has reported that electrical stimulation of carcasses has considerable effect upon development of the spoilage flora (*4*). It is difficult to explain these conclusions in a manner compatible with present knowledge of the effects of electrical stimulation on meat composition and of bacterial growth on meat. Furthermore, examination of the results presented suggest that they could bear a very different interpretation to that proposed by the authors. We have therefore examined the effect of electrical stimulation on the behavior of bacteria on meat.

MATERIALS AND METHODS

Preparation of meat samples

Six sheep carcasses were skinned and eviscerated as in normal commercial practice. Two carcasses were uninoculated. The outer surfaces of both hind legs of two other carcasses were swabbed with a suspension of *Pseudomonas fluorescens* to give an initial density of about $10^4/cm^2$. The outer surfaces of the hind legs of the two remaining carcasses were seared with a hot iron and an area of surface tissue (100 cm^2) removed from each. The exposed muscle surfaces were inoculated with *P. fluorescens* ($10^4/cm^2$). One leg was removed from each carcass and placed in a plastic bag. The carcasses were then stimulated (pulse peak 1130V, 858 pulses.min^{-1}, duration 10 ms, current 1.8A) for 90 sec at 30 min post mortem, as in normal commercial practice in New Zealand (*1*). The remaining hind legs were removed from all carcasses. Approximately 150 g of meat was removed from each uninoculated, and seared and inoculated leg. Each sample was minced separately in a sterilized mincer and portions of approximately 15 g distributed in sterile petri dishes.

Storage and sampling

The surface-inoculated legs, in plastic bags, and the minces were stored at 10 C. The legs were sampled by swabbing areas of 5 cm^2, and minces by stomaching 10-g samples with 20 ml of peptone water. Samples were suitably diluted with peptone water and spread on nutrient agar plates which were incubated at 30 C.

RESULTS AND DISCUSSION

The conditions chosen for storage and sampling have been shown to yield results which parallel the behavior of spoilage bacteria at lower temperatures (*2*). There was no difference in lag phase, growth rate or maximum cell density of the bacteria between electrically stimulated or control samples (Fig. 1). The results of Raccach and Hendrickson (*4*) show significant differences in all these respects. These are difficult to understand, as electrical stimulation produces no changes in meat which do not occur naturally (*1*). Bacteria in minces and on meat where there is no drying of the surface grow at their maximum rate for the storage temperature (*3*). It is therefore difficult to see how electrical stimulation could lead to an enhanced growth rate of the spoilage flora. Even selection of the most rapidly growing species as a result of electrical stimulation is not a valid proposal, since such selection always occurs during development of the spoilage flora (*3*), and Raccach and Hendrickson

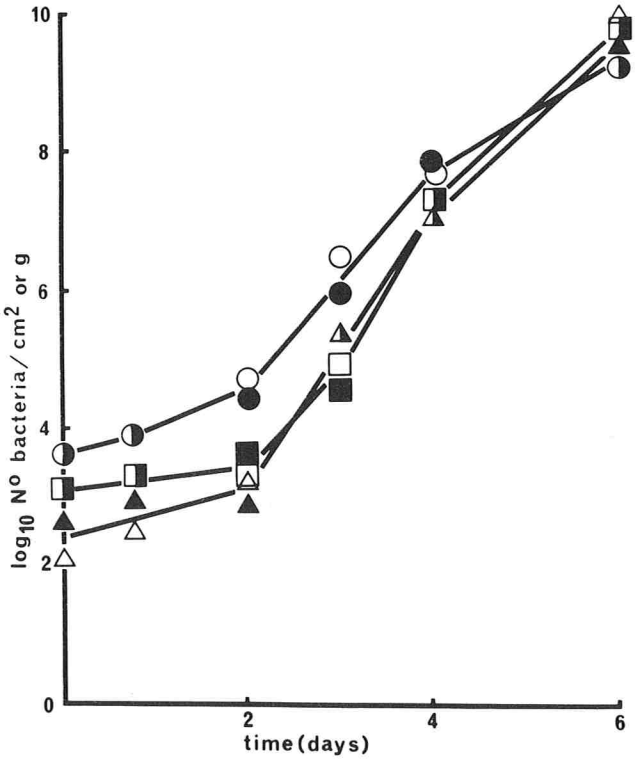

Figure 1. *Bacterial growth at 10 C on surface of mutton legs inoculated with* P. fluorescens (○), mince prepared from inoculated *legs* (□) *and mince prepared from uninoculated legs* (△). *Open symbols, unstimulated legs; solid symbols, electrically stimulated legs.*

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Inhibition of *Salmonella typhimurium* and *Staphylococcus aureus* by Butylated Hydroxyanisole and the Propyl Ester of p-Hydroxybenzoic Acid

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ABSTRACT

Inhibition of *Salmonella typhimurium* and *Staphylococcus aureus* in Trypticase Soy Broth by the propyl ester of p-hydroxybenzoic acid (propyl paraben, 0 to 500 ppm) and butylated hydroxyanisole (BHA, 0 to 400 ppm) alone and in combination was studied. A concentration of 200 ppm of BHA was bactericidal to *S. aureus*, while up to 400 ppm of BHA was only restrictive to the growth of *S. typhimurium*. A gradual decline in viable cell numbers of *S. aureus* was noted with addition of 500 ppm of propylparaben while an initial reduction of *S. typhimurium* and subsequent growth occurred at a level of 300 ppm of propylparaben. A combination of 50 ppm of BHA-propylparaben was needed to produce a gradual reduction in viable cells of *S. aureus* and 150 ppm of each additive caused *S. typhimurium* to exhibit an apparent decrease in cell numbers and then limited growth.

The ability of certain phenolic compounds to serve as antimicrobial agents in foods has been investigated (*1,4,5,6,10*). The structural similarity between butylated hydroxyanisole (BHA) and other phenolic compounds used as antimicrobial agents suggests that BHA might also be effective for this purpose. Recent studies have demonstrated BHA to be an inhibitor of bacterial growth. Chang and Branen (*2*) found that 150 ppm of BHA was necessary for inhibition of *Staphylococcus aureus* in nutrient broth and *Salmonella typhimurium* was only slightly inhibited by 400 ppm of BHA. *S. aureus* was less susceptible to BHA, requiring 400 ppm for inhibition in a study done by Shih and Harris (*8*). In the same study, 400 ppm only reduced final cell numbers of *Escherichia coli*. Growth of *Vibrio parahaemolyticus* was reported to be inhibited by 50 ppm of BHA in Trypticase Soy Broth (*6*).

Schelhorn (*7*) suggested the possibility of using a combination of food additives with the p-hydroxybenzoates so that a minimum concentration could be used for microbial inhibition. Aalto et al. (*1*) demonstrated that the effect of mixtures of methyl and propyl p-hydroxybenzoates (parabens) on *Aspergillus niger* was additive. The purpose of this study was to determine the effect of combinations of BHA and propylparaben on growth of two foodborne pathogens.

MATERIALS AND METHODS

Test organisms

The test organisms were *S. aureus* 262 procured from the U.S. Food and Drug Administration and *S. typhimurium* 3-4 from our own culture collection. Frozen stock cultures of *S. aureus* and *S. typhimurium* were used to inoculate Trypticase Soy Broth (BBL) (TSB, pH 7.0). These were incubated for 12 h at 35 C before growth studies.

Test compounds

Butylated hydroxyanisole (BHA), (Eastman Chemical Co., Kingsport, TN) was dissolved in 95% ethanol to a concentration of 2% (w/v). The solution was filter-sterilized (Millipore 0.22 μ) and stored in a sterile stoppered flask at 4 C. The propyl ester of p-hydroxybenzoic acid (propylparaben) (Mallenkrodt Inc., Lodi, NJ) was dissolved to a final concentration of 10% (w/v) in 95% ethanol, filter-sterilized and stored as above.

Growth inhibition studies

Growth studies were made in 250-ml screwtop Erlenmeyer flasks containing 50 ml of TSB. Predetermined amounts of BHA and/or propylparaben were added to the appropriate sterile medium.

A 1% inoculum of the 12-h-old culture was used for all growth experiments. The flasks were incubated at 35 C and 280 RPM in a shaker waterbath (Fermentation Design Inc., Allentown, PA, USA) for a 48-h period.

Enumeration of cells

At selected time intervals, samples from the growth flasks were taken, serially diluted in sterile 0.1% (w/v) peptone at pH 7.0 and pour-plated with Trypticase Soy Agar (BBL) (TSA). The plates were incubated at 35 C for 24 h before counts were recorded. Reported plate counts were mean values of duplicate experiments.

RESULTS

BHA inhibition

Cell numbers of *S. aureus* declined slowly in the presence of 100 ppm of BHA, while 200 ppm of BHA proved bactericidal (Fig. 1). *S. typhimurium* was less sensitive to BHA; concentrations of 100, 150 and 200 ppm progressively restricted the final cell numbers (Fig. 2). BHA at a concentration of 400 ppm produced an apparent rapid decrease in the viable cell count of *S. typhimurium* over 3 h of incubation, followed by an increase in cell numbers to a final cell count that was two log cycles greater than the initial inoculum. Control flasks containing 2% (v/v) ethanol in TSB were inoculated and incubated. The growth response of both

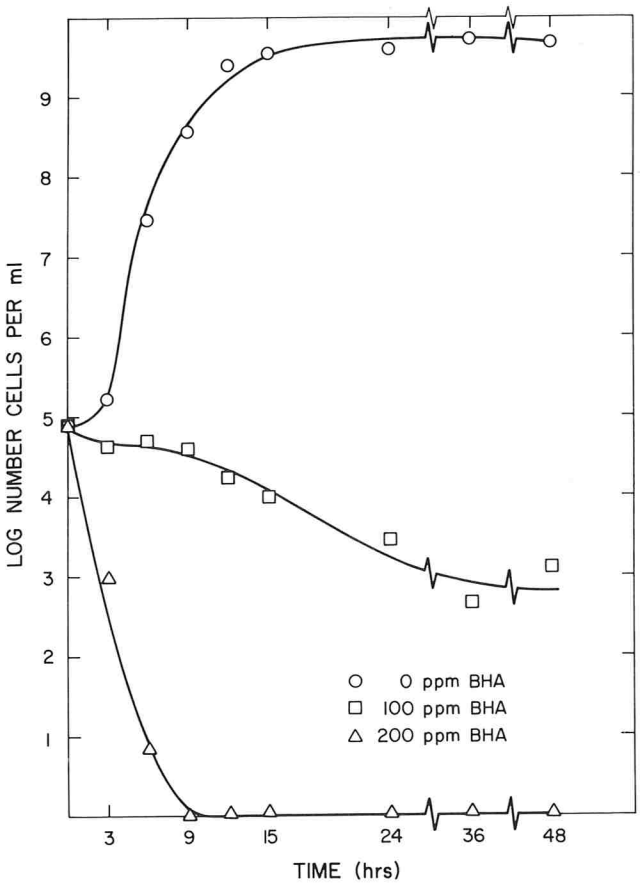

Figure 1. *Inhibition of* Staphylococcus aureus *at 35 C in Trypticase Soy Broth containing 0, 100, 200 ppm butylated hydroxyanisole (BHA).*

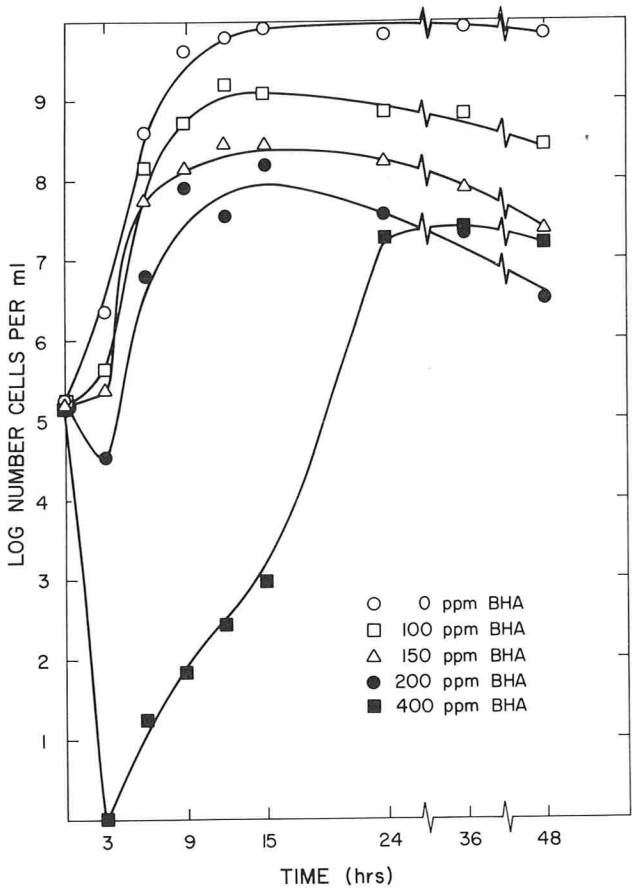

Figure 2. *Inhibition of* Salmonella typhimurium *at 35 C in Trypticase Soy Broth containing 0, 100, 150, 200 and 400 ppm butylated hydroxyanisole (BHA).*

test organisms in this medium was the same as that obtained in TSB; therefore, inhibition was not due to the ethanol added with the inhibitors.

Propylparaben inhibition

Some inhibition of *S. typhimurium* was noted as a result of the addition of 200 or 250 ppm of propylparaben to the growth medium (Fig. 3). Initial reduction of cell numbers with subsequent growth occurred when 300 ppm of propylparaben was added. *S. aureus* tolerated up to 250 ppm of propylparaben with little restriction of growth; 500 ppm of propylparaben was necessary to see a decline in the cell population (Fig. 4).

Combined inhibition

Very little reduction in growth of *S. aureus* was noted when 50 ppm of each compound was used, while addition of 100 ppm of each additive caused the number of cells in the medium to decline slowly over the 48-h period (Fig. 5). No viable cells were detected after 6 h upon addition of 150 ppm of each compound. The maximum viable cell number of *S. typhimurium* was reduced by a combination of either 75 or 100 ppm of each compound (Fig. 6). When 150 ppm of each additive was employed, the cell population exhibited an apparent decrease and then limited growth.

DISCUSSION

Considerable interest has been shown in BHA as a possible microbial inhibitor (*2,6,8*). Past studies have not made direct comparisons of the effectiveness of the parabens and antioxidants on growth inhibition of foodborne pathogens. Results of this study indicate that BHA is more effective than propylparaben in inhibiting growth of *S. aureus* and *S. typhimurium* in TSB. In combination, BHA and propylparaben showed no additional inhibition against *S. aureus* in comparison to BHA alone. However, an additive effect of the two compounds was noticeable with *S. typhimurium.* A potential use of BHA and propylparaben in combination exists in foods, since they are shown here to be effective inhibitors at levels below those currently allowed (200 ppm for BHA, 1,000 ppm for propylparaben).

The apparent decrease and subsequent increase in viable cells of *S. typhimurium* in the presence of 200 and 400 ppm of BHA (Fig. 2) has been previously reported (*2*). Other gram-negative bacteria have shown a similar response in the presence of BHA (*6,8*). The same type of growth pattern was obtained in this study with 300 ppm of propylparaben or a combination of 150-150 ppm of BHA-propylparaben (Fig. 3, 6). *Clostridium perfringens*

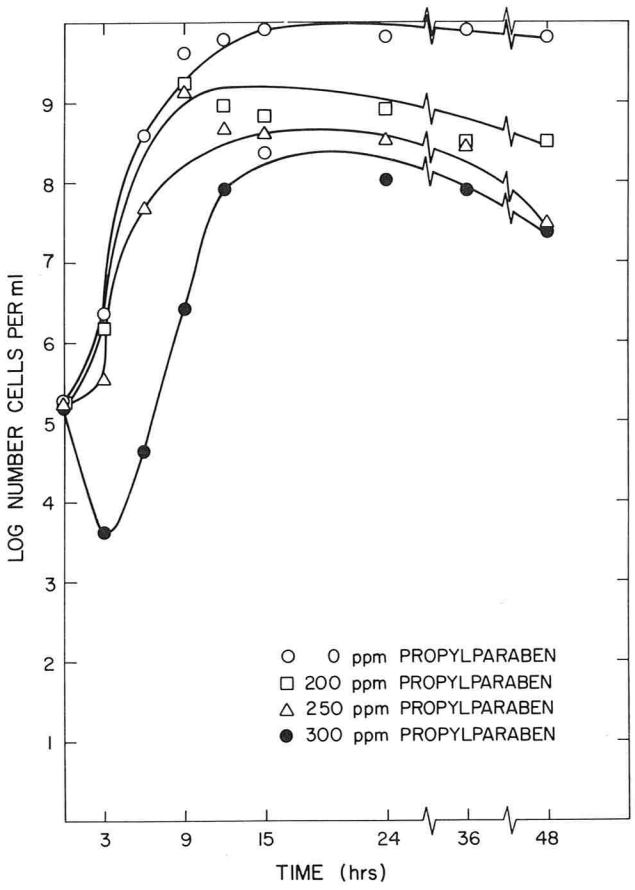

Figure 3. *Inhibition of* Salmonella typhimurium *at 35 C in Trypticase Soy Broth containing 0, 200, 250, and 300 ppm propylparaben (PP).*

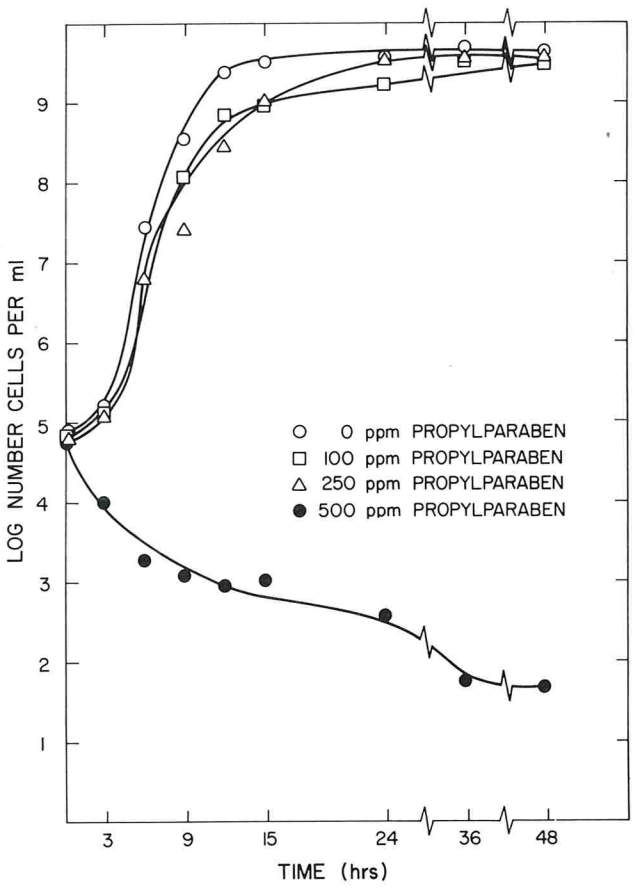

Figure 4. *Inhibition of* Staphylococcus aureus *at 35 C in Trypticase Soy Broth containing 0, 100, 250, and 500 ppm propylparaben (PP).*

exhibits a decrease in plate count, followed by a rapid increase in count that continues above the initial count when cells are heated at 50 C (*3*). Collee et al. (*3*) presented several explanations for this phenomenon that might apply to our study. However, Shoemaker and Pierson (*9*) found that this phenomenon with *C. perfringens*, termed the "Phoenix Phenomenon", was an injury-recovery process. The growth response of *S. typhimurium* to high concentrations of BHA or propylparaben could be an injury-recovery phenomenon. The mechanism of this growth pattern is currently under investigation.

The usefulness of BHA as an antimicrobial agent by itself and in combination with propylparaben may prove instrumental in the control of foodborne pathogens of public health significance. An additional benefit from the use of this food additive in combination with antimicrobial agents in foods is its antioxidant properties. Even though BHA exhibited antimicrobial activity in the laboratory media used in this study, its effectiveness in foods along with propylparaben needs to be examined. Robach et al. (*6*) found that the concentration of BHA necessary to inhibit *Vibrio parahaemolyticus* was 50 ppm in Trypticase Soy Broth while 400 ppm was necessary for growth inhibition in a crabmeat homogenate.

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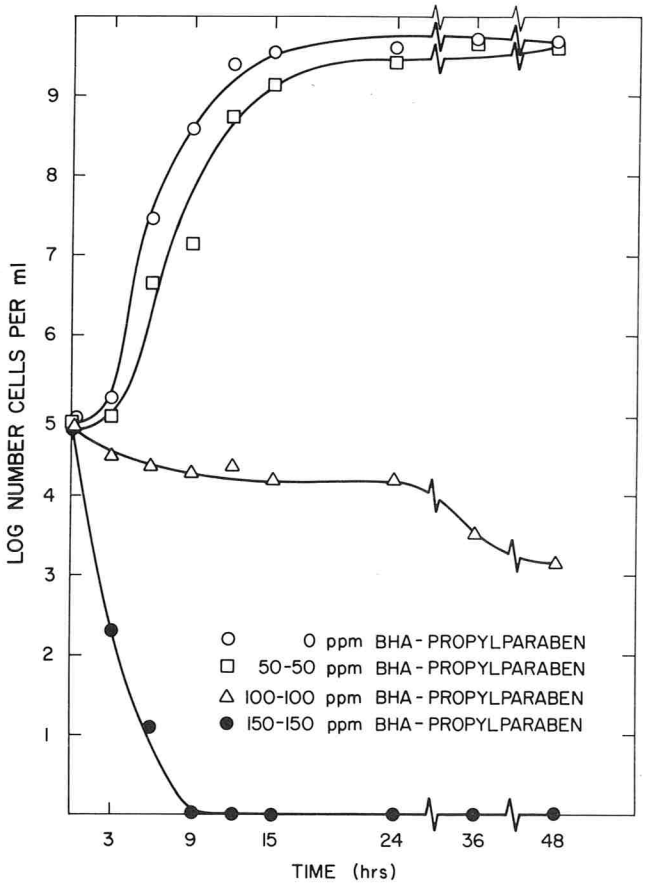

Figure 5. *Inhibition of* Staphylococcus aureus *at 35 C in Trypticase Soy Broth containing combinations of 0 ppm butylated hydroxyanisole-propylparaben (BHA-PP), 50-50 ppm BHA-PP, 100-100 ppm BHA-PP, and 150-150 ppm BHA-PP.*

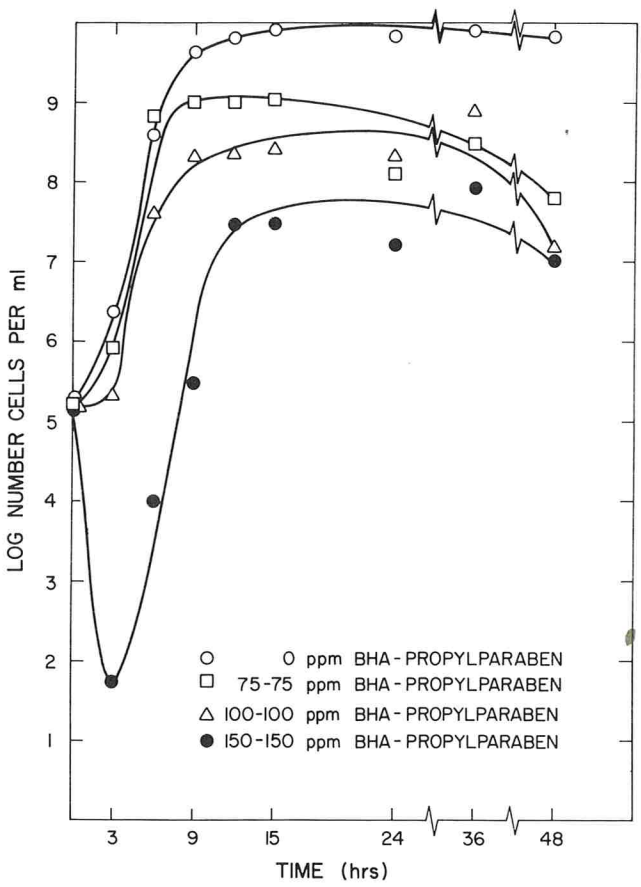

Figure 6. *Inhibition of* Salmonella typhimurium *at 35 C in Trypticase Soy Broth containing combinations of 0 ppm butylated hydroxyanisole-propylparaben (BHA-PP), 75-75 ppm BHA-PP, 100-100 ppm BHA-PP, and 150-150 ppm BHA-PP.*

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Gill, *con't from p. 190*

report that no differences were found between the flora of their minces. The differences in maximum cell density observed, but not commented upon, by Raccach and Hendrickson are also inexplicable, as under aerobic conditions maximum cell density is determined by availability of oxygen (*3*). This could not be affected by electrical stimulation. The extended lag phase observed by Raccach and Hendrickson has the merit of being possible in relation to present knowledge about bacterial growth on meat. However, they contend that their result shows a 1-day lag in the control and 3 days in the stimulated mince, whereas their figure can readily be interpreted as showing a 2-day lag in both instances. The results presented in this communication give no indication of any differences in length of the lag phase. Although the time during which stimulation was applied differed in the two studies, the time of current flow and the electrical energy input are not vastly dissimilar. It therefore seems reasonable to conclude that electrical stimulation per se has no discernible effect upon the development of meat spoilage floras.

ACKNOWLEDGMENT

I am grateful to Mr. J. C. L. Harrison for his skilled technical assistance.

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Inhibition of *Clostridium botulinum* by Spice Extracts and Aliphatic Alcohols

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ABSTRACT

Alcoholic extracts of spices were prepared and tested for inhibition of *Clostridium botulinum* in culture media. Mace (the outer covering of the seed of *Myristica fragrans*) and achiote (annato, *Bixa orellana*) were the most inhibitory of 33 spices studied. Also quite active were bay leaf (*Laurus nobilis*), white and black pepper (*Piper nigrum*) and nutmeg (the seed of *M. fragrans*). Less active were rosemary (*Rosmarinus officinalis*), clove (*Eugenia caryophyllata*), oregano (*Oreganum vulgare*), turmeric (*Circuma longa*), thyme (*Thymus vulgaris*), and paprika (*Capsicum annuum*). Of the series C_1 to C_{18}, aliphatic straight chain alcohols of C_{14} or C_{16} chain-lengths were the most inhibitory against *C. botulinum* with a minimum inhibitory concentration (MIC) of 0.6 ppm. A plot of alcohol chain length versus MIC showed a highly significant ($P < 0.01$) cubic function.

Spices are inhibitory to a number of species of aerobic bacteria. Dold and Knapp (*3*) showed garlic and onion to be active against all microorganisms tested. Nutmeg and mustard inhibited all organisms tested except *Bacillus subtilis*. Beuchat (*1*) found oregano and thyme were inhibitory to *Vibrio parahaemolyticus*. Pepper was active against *Escherichia coli* in sausage according to Salzer et al. (*12*), but they found the curing organisms, the micrococci and lactobacilli, to be unaffected by relatively high concentrations. Farbood et al. (*6*) reported that a rosemary spice extract was active against *Staphylococcus aureus* in culture media; gram negative bacteria were unaffected.

Fatty acids have been shown by several workers to possess antibacterial activity (*5,7,11*). Borick et al. (*2*) showed fatty acid salts to be capable of inhibiting a variety of bacteria. Huhtanen and Micich (*9*) showed aliphatic amines to be active in inhibiting *Clostridium botulinum*.

The literature on spice-inhibition of bacteria contains no references to studies with *C. botulinum* nor has the effect of aliphatic alcohols been reported. The studies reported here were undertaken to determine the effect of spices and a series of straight chain aliphatic alcohols, from C_1 to C_{18}, against *C. botulinum*.

MATERIALS AND METHODS

The assay method and medium were those of Huhtanen (*8*). Spices were obtained from local retail outlets. Extracts were made by steeping 10 g of a spice in 90 ml of ethanol for 48 h at room temperature with occasional stirring. A 0.1-ml portion of the undiluted, particle-free supernatant liquid of an extract was added to each of two tubes each containing 5 ml of sterile assay medium. Serial dilutions of each extract were made with sterile assay medium to contain 1/4, 1/16, 1/256 and 1/1024 the original concentration of extractives per unit volume, and 0.1 ml of each dilution was added to each of two tubes each containing 5 ml of assay medium. Extraction efficiency was not determined, but the six levels of each spice extract added to the assay tubes represented the extract equivalents of about 2000, 500, 125, 31, 8 and 2 ppm of the original spice in terms of the final contents of the assay tubes.

The C_1 to C_{12} alcohols were added in amounts of 0.2, 0.1 and 0.05 ml to 5-ml quantities of assay medium in tubes. The final concentrations of alcohols (v/v) were 40,000, 20,000, and 10,000 ppm. The alcohols were then diluted by adding 0.5 ml to 3.5 ml of assay medium. This 1:8 dilution was added to assay tubes in 0.2, 0.1 and 0.05-ml amounts, giving final concentrations of 5,000, 2,500 and 1,250 ppm. The 1:8 dilutions and additions were repeated, giving a series of $\log_2$ dilutions.

The insoluble alcohols, C_{13}, C_{14}, C_{16} and C_{18}, were made up in 10 mg/ml concentrations in ethyl alcohol, and 0.1 and 0.05 ml were added to tubes of autoclaved assay medium. The alcohols were diluted 1:8 in assay medium as above, and, though these alcohols formed suspensions, they were fine and uniform enough so they could be diluted and pipetted as with the C_1 to C_{12} alcohols.

Determination of MIC was made visually by observing turbidity. When the organism grew, turbidity was usually readily apparent, especially when viewed through transmitted light. In cases where a slight amount of growth was apparent in one tube, for example at 40,000 ppm, and maximum growth in the next dilution, 20,000 ppm, the MIC was estimated to be between the two or, in this example, 30,000 ppm. In cases where insoluble alcohols formed turbidity, verification of growth or no growth was made by observing gas production when a hot loop was plunged into tubes warmed to 50 C. In cases of questionable gas production by the hot loop method, further evaluation of growth was made by examining gram stains of the cultures.

RESULTS AND DISCUSSION

The most inhibitory spices (Table 1) were mace and achiote with a MIC of 31 ppm. Nutmeg, bay leaf and white and black pepper were also quite active with a MIC of 125 ppm. Lesser activities, with MICs of 500 ppm, were exhibited by the extracts of paprika, rosemary, cloves, oregano, turmeric and thyme. The rest of the spices showed little or no inhibition at 2,000 ppm.

The inhibition of *C. botulinum* by aliphatic alcohols is shown in Table 2. Methanol and ethanol gave a MIC of 30,000 ppm; this decreased to 0.6 ppm for tetradecanol and hexadecanol, increasing again to 25 ppm for octadecanol. The curve of chain length plotted against Ln MIC, shown in Fig. 1, shows a highly significant ($p < 0.001$) cubic function and a less significant ($p < 0.005$) quadratic function. The increase in MIC with

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TABLE 1. *Inhibition of* Clostridium botulinum *by spices.*

Name		
Common	Botanical	MIC[a] (μg /ml)
Allspice	*Pimenta officinalis*	2000
Parsley	*Petroselinum crispum*	> 2000
Marjoram	*Marjoram hortensis*	> 500
Mustard	Blend of *Brassica hirta* and *Sinapis alba*	> 2000
Garlic	*Allium sativum*	> 2000
Celery flakes	*Apium graveolens*	> 2000
Celery seed	*Apium graveolens*	2000
Chives	*Allium schoenoprasum*	> 2000
White pepper	*Piper nigrum* (water soaked)	125
Black pepper	*Piper nigrum* (dried fruit)	125
Sweet pepper	*Capsicum annuum*	> 2000
Paprika	*Capsicum annuum*	500
Anise	*Pimpinella anisum*	> 2000
Sage	*Salvia officinalis*	2000
Ginger	*Gingiber officinale*	2000
Caraway	*Carum carvi*	> 2000
Fennel	*Foeniculum vulgare*	> 2000
Achiote	*Bixa orellano*	31
Tarragon	*Artemesia dracunulus*	> 2000
Dill	*Anethum graveolens*	> 2000
Rosemary	*Rosmarinus officinalis*	500
Cinnamon	*Cinnamonum zeylanicum*	2000
Cloves	*Eugenia caryophyllata*	500
Red Pepper	*Capsicum frutescens*	> 500
Bay Leaf	*Laurus nobilis*	125
Cumin	*Cuminum cyminum*	> 2000
Oregano	*Lippia graveolens*	
	Oreganum vulgare	500
Turmeric	*Circuma longa*	500
Onion	*Allium cepa*	> 2000
Thyme	*Thymus vulgaris*	500
Nutmeg	*Myristica fragrans* (seed)	125
Mace	*Myristica fragrans* (external coat)	31
Coriander seed	*Coriandrum sativum*	> 2000

[a]10% ethanol extracts, MIC (minimum inhibitory concentrations) values are on a whole spice basis. Levels assayed were 2000, 500, 125, 31, and 8 μg/ml.

TABLE 2. *Inhibition of* Clostridium botulinum *by aliphatic alcohols.*

Alcohol	MIC μg/ml
Methanol[a]	30,000
Ethanol	30,000
Propanol	15,000
Butanol	10,000
Pentanol	5,000
Hexanol	10,000
Heptanol	600
Octanol	200
Nonanol	100
Decanol	25
Undecanol	5
Dodecanol	2.5
Tridecanol[b]	1.25
Tetradecanol	0.6
Hexadecanol	0.6
Octadecanol	25

[a]Methanol to dodecanol were diluted in assay media.
[b]Tridecanol to octadecanol were 1% solutions in ethanol with further dilutions in assay medium.

the octadecanol appears to account for the cubic trend of the curve. This curve is similar to that reported for n-alkyl esters of benzoic acid by Dymicky and Huhtanen (*4*), who found the C_{11} ester to be most active with decreasing activity to C_{18}; the curve of these results (chain length vs. Ln MIC) was also cubic.

A comparison of C_{10} to C_{18} alcohols and amines of the same chain length [data from Huhtanen and Micich (*9*)]

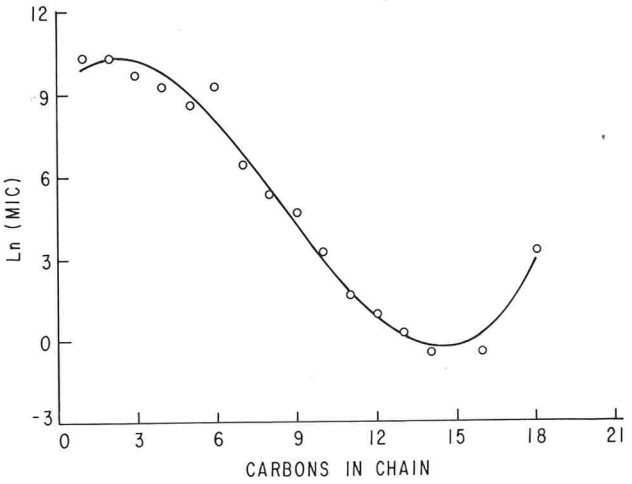

Figure 1. *Inhibition of* C. botulinum *as function of Ln (MIC) and length of aliphatic alcohol chain length.*

is shown in Fig. 2. In this range, the curve for the alcohols exhibited a highly significant binomial trend ($p < 0.01$); the curve for the amines was flatter, though still significantly ($p < 0.05$) quadratic in nature. In both studies, the C_{14} and C_{16} aliphatic amines and alcohols were the most inhibitory. Fatty acids in the range of C_9 to C_{12} (the longest chain-length studied) were found by Karabinos and Ferlin (*10*) to be highly toxic to *S. aureus*, with undecylenic acid (C_{11}) the most bactericidal. Eisler and von Metz (*5*) indicated that saturated fatty acids of C_{12}, C_{14} and C_{16} carbon atoms were more effective than the lower chain length acids in killing *Pasteurella pestis.* As in our studies with alcohols, the C_{18} fatty acid was relatively inactive.

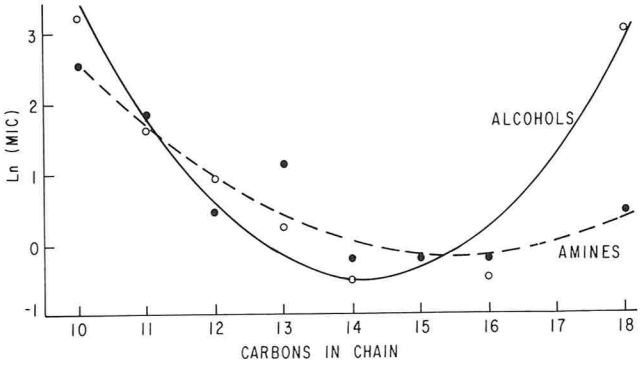

Figure 2. *Comparison of aliphatic straight chain amines and alcohols as functions of Ln (MIC) versus chain length.*

Although formulations for cured meats and sausage vary widely depending on the type of product and the manufacturer, most contain at least one or more of those spices reported here to be active against *C. botulinum* in the test tube assay. Information on exact quantities of spices used in meat products is not available from manufacturers since this is proprietary information. They are only required to list, qualitatively, the spices in their product. Achiote is not used, however, since it is a suspected carcinogen. The value of these spices as anticlostridial agents in foods has not been demon-

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Heat-Stable Proteases from Psychrotrophs in Milk

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ABSTRACT

Twelve gram-negative psychrotrophic bacteria producing heat-resistant proteases that hydrolyzed casein were isolated from refrigerated raw milk. All were pseudomonads and the enzymes of the six most proteolytic cultures were examined further. The proteases were partially purified, and gel electrophoresis indicated that only a single enzyme was present in the preparation. The molecular weight of most of the proteases was approximately 45,000. All six enzymes retained some activity after being heated at 121 C for 10 min and casein was hydrolyzed at pH levels found in normal milk and many cultured dairy products. Although proteolysis was highest at about 40 C, considerable activity was evident at refrigeration temperatures.

The trend towards extended storage of raw milk at refrigeration temperatures before processing has emphasized the significance of psychrotrophs. In the manufacture of sterile milk, the major concern has been destruction of heat-resistant spores. However, recent findings (*4,9*) suggest that the levels of heat-stable proteases produced by psychrotrophs in refrigerated raw milk are sometimes high enough to cause shelf-life problems. Most of the defects, including development of bitterness and gelation, become apparent after extended periods of storage. Mayerhofer et al. (*10*) studied a heat-stable protease produced by *Pseudomonas fluorescens* P26 which required about 9 min at 120 C for complete inactivation. Psychrotrophs capable of producing heat-stable proteases have been found in raw milk by Adams et al. (*1*). Patel et al. (*11*) isolated a psychrotrophic yeast which produced a proteolytic enzyme that rapidly hydrolyzed casein and retained significant residual activity after heating for 10 min at 100 C. White and Marshall (*16*) reported that proteases had no effect on butter quality, but Cheddar cheese and cottage cheese made from milk inoculated with a heat-resistant protease developed a bitter flavor.

The objectives of this study were to investigate heat-resistant proteases produced by psychrotrophic bacteria in raw milk, and to determine some of the characteristics of the enzymes.

MATERIALS AND METHODS

Isolation of psychrotrophs from raw milk

Raw milk from individual dairy herds was plated with Plate Count Agar (PCA) containing 1% skim milk powder. Plates were incubated at 7 C for 10 days and colonies indicating proteolysis were selected and purified by repeated streakings and incubation at 7 C. Six of the most proteolytic cultures were examined using the diagnostic tables of Cowan and Steel (*5*) and *Bergey's Manual of Determinative Bacteriology* (*2*). All isolates were found to be gram-negative rods of the genus *Pseudomonas* capable of growing from 0 to 32 C, but not at 37 C. They liquefied gelatin, but differed from each other on the basis of the extent of casein hydrolysis.

Production of proteases

The isolates were grown in sterile reconstituted skim milk (10% T.S.) at 7 C and then 0.1 ml of each culture was inoculated into 200 ml of autoclaved reconstituted skim and incubated at 7 C for 4 weeks. The incubated cultures were then centrifuged at 20,000 × *g* for 15 min at 5 C in a Sorvall RC2-B centrifuge. The supernatant fluid was dialyzed against distilled water for 24 h at room temperature and then against phosphate buffer (pH 7.2) for an additional 24 h. The dialyzate was filter-sterilized (pore size 0.45 μ) and used as a crude enzyme preparation. Partially purified enzymes were obtained by gel filtration at room temperature through Sephadex G-100 in a 40 × 2.5-cm column equilibrated with 0.02 M phosphate buffer (pH 7.2). After eluting with phosphate buffer at a flow rate of 25 ml/h, the fractions were collected, filtered through a membrane filter (pore size 0.45 μ), tested for protease activity and stored at 7 C.

Enzyme assay

Proteolytic activity was determined according to the procedure of Hull (*7*), using the Folin-Ciocalteau reagent. The substrate was 2% casein (Sigma Chemicals Co.,) in 0.02 M phosphate buffer, adjusted to the desired pH. The reaction mixture consisted of 5.95 ml of substrate and 0.05 ml of enzyme filtrate. Enzyme activity was assayed at 40 C and the reaction was stopped by adding 10 ml of 0.72 N trichloroacetic acid (TCA). Controls were prepared by immediately mixing the substrate and enzyme with TCA. Absorbance was read at 650 nm and equivalents of tyrosine released were determined from a standard curve. One protease unit is defined as the amount of enzyme that produced 1 μg of acid-soluble tyrosine per ml of enzyme solution per 24 h at 40 C.

Effect of pH and temperature on protease activity

The buffers used were 0.02 M concentrations of citrate-phosphate (pH 5.0 to 5.4), phosphate buffer (pH 6.0 to 7.8), and Tris-HCl (pH 8.4 to 9.0). The extent of proteolysis in 1 h at 40 C was determined by the Hull method.

The effect of temperature on activity was measured in the range of 4 to 55 C. The assays were performed in 0.02 M phosphate buffer (pH 6.8).

Heat treatments

Tests for inactivation of enzymes were conducted in sealed thermal death time tubes containing 5 ml of the enzyme filtrate. The tubes were immersed in a controlled oil bath of different temperatures for various time periods. The heat-up time for all samples was 7 min, as measured by a copper-constantan thermocouple sealed in one tube. The heated samples were cooled in ice water and the remaining activity was measured within 10 min after each heat treatment. All values were expressed as percent of the activity of the unheated control.

Polyacrylamide gel electrophoresis

The partially purified proteases were examined by disc gel electrophoresis according to the procedure of Davis (*6*). Electrophoresis was performed in 7.5% gels and Tris-glycine buffer (pH 8.9) at a

current of 4 mA/gel. Experiments were carried out at 5 C and terminated after about 2 h when the brom-phenol blue marker reached the bottom of the gel. After electrophoresis, one set of the gels was stained for 1 h at 37 C with 0.1% Coomassie Brilliant Blue R-250 solution made up freshly in 50% TCA. The gels were destained in 7% acetic acid. The other set was assayed for proteolytic activity by cutting the gels longitudinally and placing them on PCA containing 1% skim milk powder. Clear zones were visible after 18 h of incubation at 40 C.

Molecular weight determination

Molecular weights were determined by sodium dodecylsulfate polyacrylamide gel electrophoresis in 10% gels according to the procedures of Weber and Osborn (*15*) and Laemmli (*8*).

RESULTS AND DISCUSSION

One of the methods for extending shelf-life of fluid milk without refrigeration is Ultra High Temperature (UHT) processing. However, the presence of heat-resistant enzymes could be an obstacle to the success of this process. In this study, all isolates produced proteolytic enzymes that remained active after heating at 100 C for 30 min. The proteases could be differentiated by the size of the clear zones they produced when filter paper discs, saturated with enzyme preparations, were placed on surfaces of PCA containing 1% skim milk powder. Of the twelve psychrotrophs isolated, the six most proteolytic were maintained in sterile milk at 7 C and examined further.

Effect of heat treatment on the proteases

The enzyme solutions were heat-treated at 72, 100 and 121 ± 0.5 C for times ranging from 2 to 30 min. All of the proteases differed in their heat stabilities although they had similar inactivation profiles. Representative heat-inactivation curves for 3 typical enzymes are shown in Fig. 1, 2 and 3. The proteolytic enzyme of isolate 22 (Fig. 1) was the most heat-resistant and retained 74% of its original activity after heating for 30 min at 72 C and 10% after 10 min at 121 C. Although not reported, the proteases of isolates 4, 12 and 20 showed similar results. Isolate 23 (Fig. 2) produced an enzyme that retained about 7% of its activity after heating for 8 min at 121 C. The protease of isolate 16 (Fig. 3) was the least heat-resistant with only 12% activity left after 6 min exposure at 121 C.

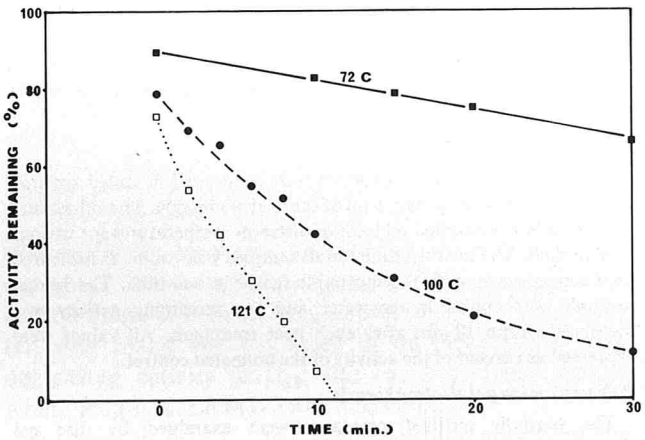

Figure 1. *Effect of heating time at 72, 100 and 121 C on enzyme activity of culture No. 22 (pH 6.8, at 40 C).*

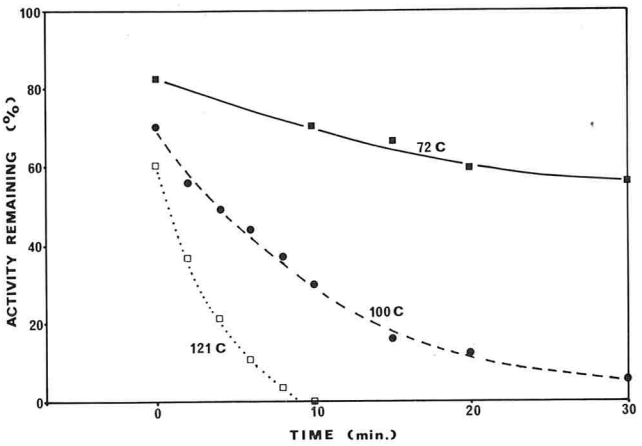

Figure 2. *Effect of heating time at 72, 100 and 121 C on enzyme activity of culture No. 23 (pH 6.8, at 40 C).*

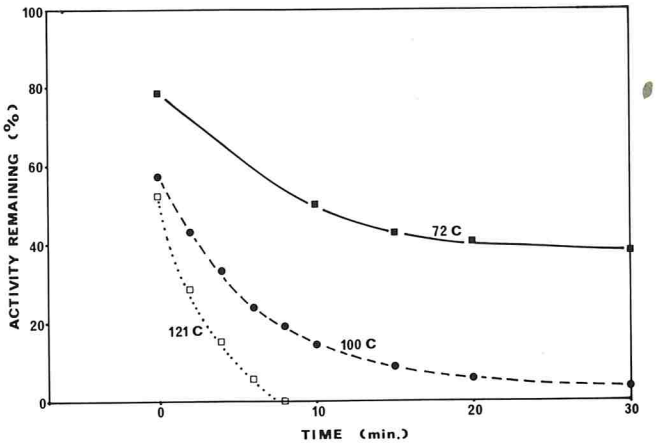

Figure 3. *Effect of heating time at 72, 100 and 121 C on enzyme activity of culture No. 16 (pH 6.8, at 40 C).*

The time required for the solutions to reach the desired temperatures was not included in the holding time. However, the 7-min heat-up time, particularly at the highest temperature, caused a considerable loss in the activity of all the proteolytic enzymes.

Heat treatments of 120 to 150 C for 1 to 8 sec have been proposed for sterilizing milk (*3,13,14*). However, results in the present study and at the enzyme concentrations used showed that heating at 121 C for 2 min would destroy less than 40% of most of these enzymes. Adams et al. (*1*) reported that the proteases of some *Pseudomonas* species isolated from raw milk retained more than 70% of their activity during sterilization of milk at 149 C for 10 sec.

Ideally, one could increase the severity of heat treatments to control these enzymes by either increasing the temperature or extending the holding time. However, any UHT treatment that would completely destroy the proteases would also have an adverse effect on the product. Heating milk at 72 C (HTST pasteurization) would require much longer treatment times, resulting in flavor defects.

The effect of pH on the activity of the proteases was evaluated at pH values ranging from 5.0 to 9.0. Each

sample appeared to have a distinctly different proteolytic system. Data from three enzyme preparations are shown in Fig. 4. Samples 23 and 4 had their maximum proteolytic activities at pH 6.5 and 7.2, respectively, while sample 22 was most active at pH 6.0. Although the optimum of most of the proteases examined was at pH 6.5 to 6.8, the pH of normal milk, it is significant that these enzymes showed 65 to 75% of their maximum activity at pH 5.0, the pH of many cultured dairy products.

Results in Fig. 5 show the effect of temperature on the relative activity of the proteases. The optimum temperature for casein hydrolysis was found to be 40 C. Above this temperature, activity declined quickly with no proteolysis being detected above 60 C. Although all the proteases examined were most active at 40 C, 60 to 80% of their maximum activity was still evident at room temperature (25 C). This is important since UHT-treated milk is usually stored without refrigeration.

It is of practical significance also, that all had a considerable activity at 4 to 7 C (e.g. isolate 23, Fig. 4), a temperature at which both raw and pasteurized milk are commonly held.

Molecular weights

The homogeneity of the proteases was analyzed with disc gel electrophoresis and dodecylsulfate gel electrophoresis. In each case, a single protein band was observed suggesting that only one protease was present in the enzyme preparation. A typical example is shown in Fig. 6.

The molecular weights of the proteases were examined by polyacrylamide gel electrophoresis containing 0.1% sodium dodecylsulfate and 0.1% mercaptoethanol. The molecular weights were estimated by comparing the migration with other proteins of known molecular weights electrophoresed under the same conditions: phosphorylase A (94,000), bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (21,500) and lysozyme (14,300). As shown in Fig. 7, the molecular weights of most of the proteases were found to be approximately 45,000 (46,000 for isolate 22, 45,000 for isolate 12 and 44,000 for isolates 4, 23 and 20). The molecular weight of isolate 16 was 22,000.

Psychrotrophic *Pseudomonas* species which produce extracellular proteases differing in heat stabilities, moleuclar weight and other properties were reported by several authors (*1,10,12*). Of the different enzymes characterized, the heat inactivation parameters of *Pseudomonas fluorescens* P26 reported by Mayerhofer et al. (*10*) closely resemble the proteases studied in this

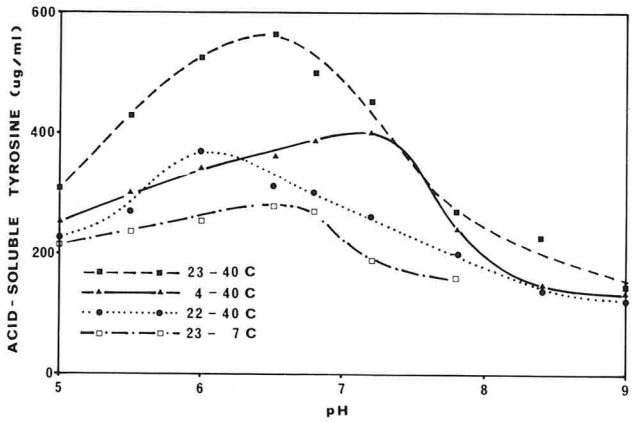

Figure 4. *Effect of pH on enzyme activity of cultures No. 23 (at 40 C and 7 C), 4 (at 40 C), and 22 (at 40 C).*

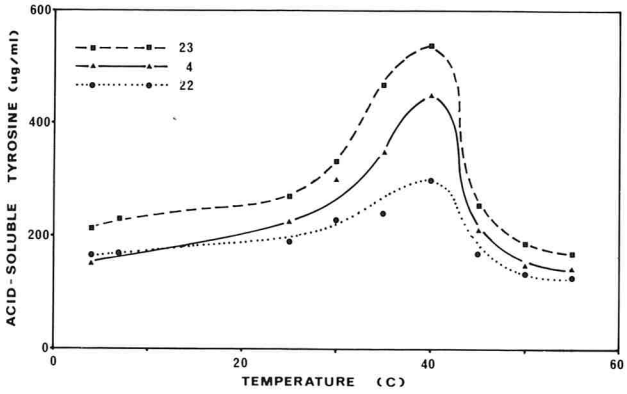

Figure 5. *Effect of temperature on enzyme activity of cultures, 23, 4, and 22 (pH = 6.8).*

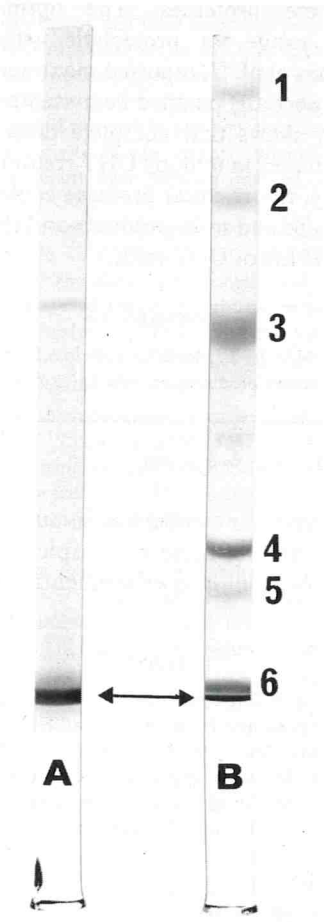

Figure 6. ***SDS-polyacrylamide gel electrophorograms of: A - Enzyme** of culture No. 22. B - Standard proteins (1 = phosphorylase B; 2 = bovine serum albumin; 3 = ovalbumin; 4 = carbonic anhydrase; 5 = soybean trypsin inhibitor; and 6 = lysozyme). Arrow indicates position of dye front.*

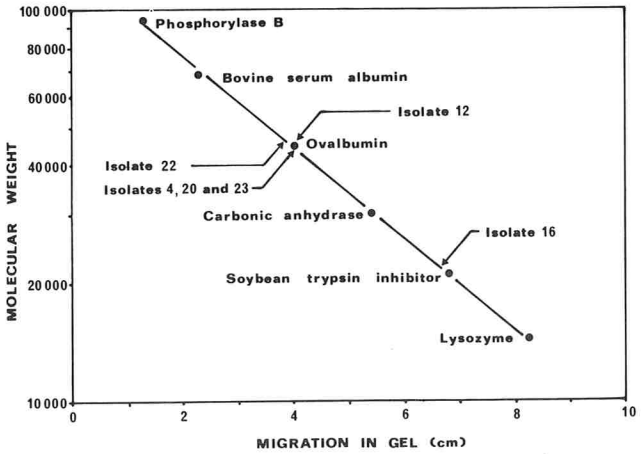

Figure 7. *Molecular weights of enzymes as determined by SDS-polyacrylamide gel electrophoresis. Standard proteins (25 μg) and enzyme solutions treated with 1% SDS were used and the electrophoresis was performed at pH 8.3 with 8 mA per tube for 3.5 h.*

investigation. However, the molecular weight of the enzyme of P26 was only approximately 23,000, which is about half of most of these proteases. The proteolytic enzyme of *Pseudomonas fragi* examined by Porzio and Pearson (*12*) had the same apparent molecular weight as most of these proteases. The optimum pH and temperature range for proteolytic activity were also similar. Adams et al. (*1*) reported maximum activity to be at 45 C for a partially purified heat-stable protease.

This study shows that complete destruction of heat resistant proteases in milk by UHT treatment is difficult. Consequently, the residual protease could cause quality changes in milk and milk products and thus shorten the expected shelf-life of UHT milk.

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strated. Research now underway in our laboratories will establish the value of these active spices and alcohols in inhibiting *C. botulinum* in experimental meat systems.

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Inactivation of Staphylococcal Thermonuclease by an Enzyme-Like Factor Produced by *Streptococcus faecalis* subsp. *liquefaciens*

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ABSTRACT

The thermonuclease of *Staphylococcus aureus* was inactivated during incubation with filtrates from cultures of *Streptococcus faecalis* subsp. *liquefaciens*. Of 53 isolates, 40 produced a thermonuclease-inactivating factor (TIF). The TIF was heat-labile, non-dialyzable and optimally active at 55 C and pH 7.0. These characteristics suggest TIF is a proteolytic enzyme. The common occurrence of TIF in cultures of *S. faecalis* subsp. *liquefaciens* may limit the value of the thermonuclease test for foods containing large populations of this organism.

The heat-stable nuclease (thermonuclease, TNase) produced by *Staphylococcus aureus* in culture media and in foods has been suggested as an indicator of *S. aureus* growth and potential public health hazards associated with the presence of staphylococcal enterotoxins (*2,5,6,7,8,10*). For thermonuclease to serve as a reliable indicator, however, it must be capable of surviving the same stresses in the food environment as those tolerated by the enterotoxins. The remarkable heat-stability of thermonuclease has been well documented (*2,3,4,8*) and the enzyme has been shown to remain active in foods undergoing thermal processing. Other stresses, however, include possible interactions with co-existing microflora within the foods, perhaps leading to inactivation of thermonuclease, or interference with detection of thermonuclease activity.

Several workers have attempted to evaluate the susceptibility of thermonuclease to inactivation by several proteolytic organisms commonly encountered in foods. Lachica et al. (*8*) investigated the effects of growing a variety of bacteria in broth containing purified thermonuclease. Of the organisms tested, only a culture of *Streptococcus faecalis* subsp. *liquefaciens* produced a significant loss in thermonuclease activity. It was not determined whether the inactivation was due to proteolysis or formation of a thermonuclease inhibitor.

The present study was conducted to determine the frequency of TNase-inactivating strains of *S. faecalis* subsp. *liquefaciens* and the nature of the inactivating substance.

MATERIALS AND METHODS

Cultures

S. faecalis isolates were obtained from culture collections, food samples, human sewage and fecal specimens from domestic animals, using a preliminary selective enrichment in KF Streptococcal broth (BBL Laboratories) followed by selective plating on Mead Agar (BBL) prepared as a dual layer plate. Typical colonies of *S. faecalis* were further characterized by gram-staining, testing for catalase and testing for the ability to grow at 4 and 45 C in Brain-Heart Infusion broth. Identification of isolates as *S. faecalis* subsp. *liquefaciens* was confirmed by observing proteolysis of gelatin (nutrient gelatin) and casein (litmus milk, Difco). All cultures were maintained on Trypticase-Soy agar slants which were stored at 4 C.

Production and recovery of thermonuclease-inactivating factor (TIF)

Isolates were inoculated into 15-ml portions of Trypticase-Soy broth in 25-ml Erlenmeyer flasks, and were incubated at 37 C for 72 h on a rotary shaker (New Brunswick) set at 150 rpm. Culture supernatant fluids were recovered by centrifugation at 10,000 × *g* for 20 min using a Sorvall RC-2B refrigerated centrifuge. The recovered supernatant fluids were concentrated five-fold by pervaporation and were stored at -20 C. Proteolytic activity in the culture supernatant fluids was confirmed using a gelatin-agar diffusion method, as described by Madler et al. (*9*).

A partial purification of TIF was achieved by bringing 500 ml of a 72-h supernatant fluid to 75% saturation with ammonium sulfate (Baker, reagent grade) and allowing precipitation to occur for 24 h at 4 C. The precipitate was recovered by centrifugation at 8,000 × *g* at 4 C for 40 min, and was dissolved in 20 ml of distilled water. The solution was dialyzed continuously at 4 C for 24 h against distilled water.

Assay and purification of thermonuclease

Partially purified thermonuclease was prepared from a culture supernatant fluid of *S. aureus* FDA #262 by the procedure of Alexander et al. (*1*) using successive precipitations with ammonium sulfate and 3 N trichloracetic acid. The resulting solution (TNase stock) contained 4.1 mg protein/ml and had a specific activity of 24.4 U/mg protein.

Thermonuclease was assayed by the method of Lachica et al. (*6*), using toluidine blue-O/DNA agar prepared with salmon-sperm DNA (Calbiochem; highly polymerized). Glass petri dishes (100 × 15 mm) were filled with 13.0 ml of the agar, and wells (4.8-mm outer diameter) having a 25 µl capacity were formed by removal of agar plugs with an aspirator connected to a cork borer. For determination of thermonuclease activity, wells were filled to convexity with 25 µl of sample, usng an Eppendorf pipette. Plates were incubated at 37 C for 48 h. Pink zones of DNA hydrolysis were measured with a millimeter scale, and sample potencies were interpolated from a standard curve (zone size vs. stock TNase dilution).

Screening for TIF

Equal volumes of TNase stock, diluted 1:1000 in distilled water, and concentrated streptococcal supernatant fluids were combined in wells (0.5-ml capacity) of a plastic microtiter plate (Cooke Engineering). Plates were tightly sealed with a layer of parafilm and incubated at 37 C for 24 h. Residual thermonuclease activity was determined by the agar-diffusion method described above. Each sample was assayed in duplicate.

Characterization of TIF

Thermal stability of TIF was determined by heating a portion of the partially purified material at 96 C for 15 min. Heated samples were then combined with diluted TNase stock as described earlier, using unheated samples as controls.

Inactivation of TNase by TIF was investigated at several temperatures. A 1:40 dilution of TNase stock was prepared in McIlvaine's Buffer, pH 7.0, and distributed among plastic tissue culture tubes (Falcon). Tubes containing 0.8 ml of 1:40 TNase were heated at each temperature (4, 28, 37, 55, 60 or 70 C) for 20 min before addition of 0.2 ml of partially purified TIF solution. Control tubes were prepared as above, substituting 0.2 ml of distilled water for the TIF solution. Samples were removed after 15 min incubation at each temperature and assayed for residual thermonuclease.

The effect of pH was studied by adding 0.2 ml of TIF solution to 0.8 ml of a 1:40 dilution of TNase stock prepared in McIlvaine's Buffer at pH 4.0, 5.0, 6.0, 7.0 or 8.0. All tubes were heated to 55 C before addition of TIF solution. Samples were removed after 15 min and assayed for residual thermonuclease.

RESULTS AND DISCUSSION

A total of 53 Trypticase-soy broth supernatant fluids recovered from cultures of *S. faecalis* subsp. *liquefaciens* were tested for the ability to inactivate thermonuclease. Of these, 40 (ca. 75%) produced a decrease in thermonuclease activity after incubation for 24 h at 37 C. The extent of thermonuclease inactivation varied greatly among the TIF-containing supernatant fluids, ranging from a 20% loss in activity to virtually total inactivation. Of the 40 TIF-containing supernatants fluids, 10 produced a 90% reduction in thermonuclease activity.

The activity of a partially purified TIF preparation was abolished during heating at 96 C for 15 min. A dialyzed sample of the unheated preparation retained full activity, indicating that TIF is of high molecular weight.

The effect of incubation temperature on thermonuclease inactivation by TIF is illustrated in Fig. 1. At pH 7.0, there were no observable losses in thermonuclease activity at 4, 28, or 37 C after 15 min. At 45 C, however, the reaction proceeded rapidly to produce a 97% reduction in activity. The most rapid inactivation occurred at 55 C, resulting in nearly a total loss of thermonuclease activity within 15 min. Incubation at 60 or 70 C resulted in a pronounced loss in the ability to inactivate thermonuclease, probably reflecting thermal denaturation of TIF.

The effect of pH on thermonuclease inactivation by TIF was evaluated at 55 C at pH 4.0, 5.0, 6.0, 7.0 and 8.0 using McIlvaine's buffer (Fig. 2). Only a slight loss in thermonuclease activity (ca. 9%) was observed at pH 4.0. The rate of inactivation steadily increased at pH 5.0 and 6.0, with most extensive losses in activity occurring at pH 7.0.

CONCLUSIONS

The conjecture that TIF is a proteolytic enzyme rather than an enzyme inhibitor is supported by the results of this study. TIF is a heat-labile, non-dialyzable substance exhibiting pH and temperature optima for inactivation of staphylococcal thermonuclease.

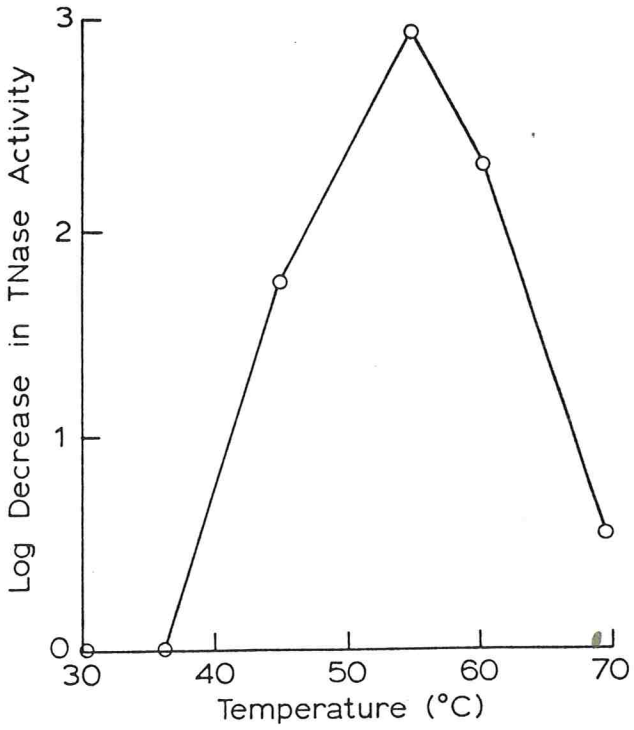

Figure 1. *Effect of incubation temperature on thermonuclease inactivation. Reaction mixture (TNase stock + partially purified TIF) was incubated at pH 7.0 (McIlvaine's buffer) for 15 min before assay.*

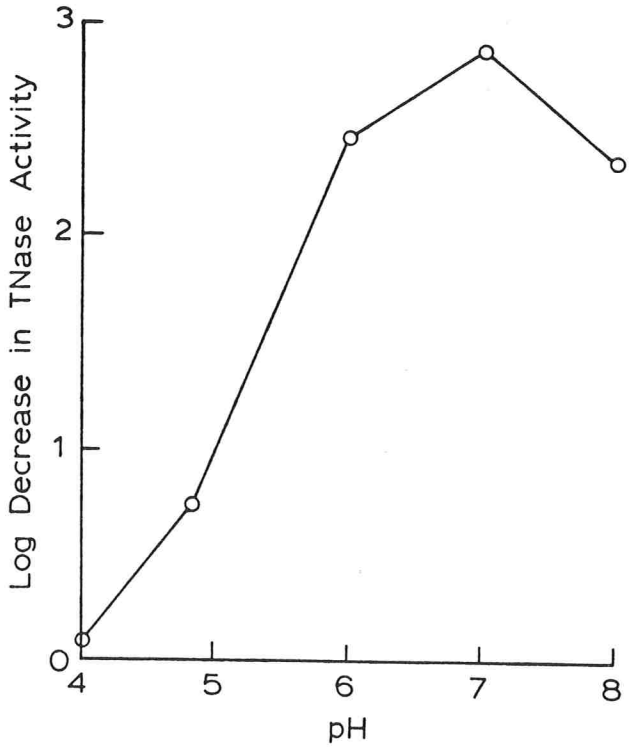

Figure 2. *Effect of pH on thermonuclease inactivation. The reaction mixture (TNase stock + partially purified TIF) was incubated at 55 C in McIlvaine's buffer for 15 min before assay.*

The relatively high frequency of proteolytic *S. faecalis* subsp. *liquefaciens* isolates capable of inactivating thermonuclease suggests that such strains may be encountered in foods, either as post-processing contami-

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A Research Note

Rodent Excreta Contamination and Insect Damage of Wheat

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ABSTRACT

A survey was made to determine the levels of insect damage and rodent excreta pellets in wheat. The analytical data obtained represented the various grade designations of wheat normally encountered during wheat grade certification in the 34 Agricultural Marketing Service, Grain Division, field offices. The mean and range of weights of insect-damaged kernels per 100 g and rodent excreta pellets and pellet fragments per kilogram were 71.5 mg (0-3809 mg) and 0.9 mg (0-100 mg), respectively. The mean and range of numbers of insect-damaged kernels and rodent excreta pellets were 3.3 (0-169) and 0.1 (0-11), respectively. The percentages of samples containing insect-damaged kernels and rodent excreta were approximately 35 and 7%, respectively.

During 1950 and 1951 the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) conducted a cooperative investigational program to determine the incidence of rodent and insect contamination in wheat and to study the relationship of insect-damaged wheat to the levels of insect contaminants in flour. As a result of this program, the regulatory limit for rodent pellets in wheat was set at one or more pellets per pint, and in September 1952, enforcement inspections and analyses were begun. In May 1953, the enforcement program was suspended and a joint committee appointed by the USDA and the Department of Health, Education, and Welfare (HEW) began a study of the grain sanitation problem. As a result of the committee's deliberations and review of data, the enforcement inspections were resumed in January 1955, with a regulatory limit of two or more rodent pellets per pint and 2% or more insect-damaged kernels.

In July 1956, the regulatory limits were lowered to one or more rodent pellets per pint and 1% or more insect-damaged kernels. After 16 years of enforcement under the 1956 regulatory limits, a joint FDA/USDA study was conducted to develop data on the levels of rodent excreta and insect damage in wheat. The sampling and analytical details of that program are presented in this report.

MATERIALS AND METHODS

Samples were collected by USDA grain inspectors from 34 field offices of the Agricultural Marketing Service, Grain Division. A total of 1200 samples was submitted by the field offices to the FDA at a rate of 100 samples per month for a 1-year period. The samples represented the various grade descriptions of wheat normally encountered in each field office jurisdiction.

Analyses were conducted by FDA analysts. Samples weighing 1000 g were analyzed for rodent (rat and mouse) excreta pellets and pellet fragments according to *Microanalytical Manual* (*1*), using method M3A1 (a) (updated October 1962). Rodent excreta was confirmed by the presence of striated hairs in the pellet or pellet fragment. Samples weighing 100 g were analyzed for insect damage according to *Microanalytical Manual* (*1*), using method M3A1 (b) (updated October 1962). Data were obtained on 1,166 samples.

RESULTS AND DISCUSSION

Wheat examined during this survey was found to contain various amounts of insect-damaged kernels and rodent excreta pellets and pellet fragments.

Table 1 presents a frequency distribution of weights of insect-damaged kernels in 100-g samples of wheat. The weight of insect-damaged kernels ranged from 0 to 3,046 mg. Approximately 35% of the wheat examined contained insect-damaged kernels. The median weight of insect-damaged kernels was 0.0 mg; the mean weight was 71.5 mg.

Table 2 presents a frequency distribution of the numbers of insect-damaged kernels in 100-g samples of wheat. The number of insect-damaged kernels ranged from 0 to 169. The median number of insect-damaged kernels was 0; the mean was 3.3.

Table 3 presents a frequency distribution of the weights of rodent excreta pellets and pellet fragments in 1000-g samples of wheat. The weight of whole pellet and

TABLE 1. *Frequency distribution of weight of insect-damaged kernels in 100-g samples of wheat.*

Weight of insect-damaged kernels (mg)	Number of samples	% Samples	Cumulative %
3000 - 3046	1	0.1	0.1
2000 - 2999	6	0.5	0.6
1000 - 1999	12	1.0	1.6
900 - 999	1	0.1	1.7
800 - 899	0	0.0	1.7
700 - 799	3	0.3	2.0
600 - 699	7	0.6	2.6
500 - 599	1	0.1	2.7
400 - 499	10	0.9	3.6
300 - 399	15	1.3	4.9
200 - 299	16	1.4	6.3
100 - 199	71	6.0	12.3
1 - 99	259	22.2	34.5
0	764	65.5	100.0

TABLE 2. *Frequency distribution of number of insect-damaged kernels in 100-g samples of wheat.*

Number of insect-damaged kernels	Number of samples	% Samples	Cumulative %
101 - 169	5	0.4	0.4
91 - 100	4	0.3	0.7
81 - 90	2	0.2	0.9
71 - 80	3	0.3	1.2
61 - 70	2	0.2	1.4
51 - 60	2	0.2	1.6
41 - 50	3	0.3	1.9
31 - 40	5	0.5	2.4
21 - 30	8	0.7	3.1
11 - 20	30	2.6	5.7
1 - 10	338	28.9	34.6
0	764	65.4	100.0

TABLE 3. *Frequency distribution of weights of rodent excreta pellets and pellet fragments in kg samples of wheat.*

Weight of excreta pellets & pellet fragments (mg)	Number of samples	% Samples	Cumulative %
100	1	0.1	0.1
51 - 60	2	0.2	0.3
41 - 50	1	0.1	0.4
31 - 40	0	0.0	0.4
26 - 30	2	0.2	0.6
21 - 25	5	0.4	1.0
16 - 20	10	0.9	1.9
11 - 15	10	0.9	2.8
6 - 10	25	2.1	4.9
1 - 5	21	1.8	6.7
0	1089	93.3	100.0

pellet fragments ranged from 0 to 100 mg. Approximately 7% of the wheat examined contained rodent excreta. The median weight of rodent excreta was 0.0 mg; the mean weight was 0.9 mg.

Table 4 presents a frequency distribution of the numbers of rodent excreta pellets in 1000-g samples of wheat. The number of rodent excreta pellets ranged from 0 to 5. The median number of excreta pellets was 0; the mean was 0.1.

Table 5 provides a statistical summary of both weights and numbers of insect-damaged wheat and rodent excreta pellet contamination.

TABLE 4. *Frequency distribution of numbers of rodent excreta pellets in kg samples of wheat.*

Number of excreta pellets	Number of samples	% Samples	Cumulative %
5	1	0.1	0.1
3	2	0.2	0.3
2	11	0.9	1.2
1	46	3.9	5.1
0	1106	94.9	100.0

TABLE 5. *Statistical summary for defect variables in wheat.*

	Rodent excreta per kilo		Insect damaged kernels per 100 g	
	Weight (mg)	Number (pellets)	Weight (mg)	Number
Median	0	0	0	0
Mean	0.89	0.10	71.48	3.28
Standard[a] deviation	5.11	0.50	283.88	13.68
Minimum value	0	0	0	0
Maximum value	100.00	5.00	3046.00	169.00

[a]Assuming normal distribution.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the work of the USDA inspectors who collected the samples and the FDA analysts who analyzed the samples. A special acknowledgment is made to John C. Atkinson for statistical analysis of the data.

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nants, or as part of the native microflora. The degree of thermonuclease inactivation effected by the isolates varied; however, any loss in thermonuclease activity may be undesirable when only low levels are present in foods being screened for the presence of *S. aureus*. Therefore, the thermonuclease test might not serve as a reliable indicator of prior growth of *S. aureus* in food if: (*a*) *S. faecalis* subsp. *liquefaciens* also had been present in the food, (*b*) TIF had been produced and (*c*) the proper environmental conditions had obtained in the food to allow expression of TIF activity.

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Scanning Electron Microscopy and Etiological Studies of Teat Cup Inflations for Mastitis Control

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ABSTRACT

Scanning electron microscopy was utilized in evaluating inner surfaces of rubber and silastic® teat cup inflations. New inflations and inflations subjected to 1000 and 5000 or more milkings were examined. Cracks and depressions appeared in new rubber inflations and increased in width (from 1.0 μm to 15.0 μm) with use. No cracks were visible in the silastic® product. Bacteria appeared to be present in the cracks of the inner surface of used rubber inflations but were not present in silastic®.

Teat cup inflations and their relationship to mastitis have long been the subjects of consideration in control of mastitis. Basic inflation design relative to (*a*) pressure differentials across the teat orifice, (*b*) bore size of the inflation, (*c*) flooding, (*d*) inflation venting and (*e*) sanitation have been considered (*3,4,5,6,7,8,9,10,11*). The back-flow of milk against the teat orifice is another reason for the spread of mastitis.

Bacteria-laden milk droplets can be introduced into the teat by pressure changes within the milking machine, particularly if the teat end is damaged. Firstenberg-Eden et al. (*2*) demonstrated that bacteria can remain attached to the skin of teats locked in small holes or in the skin tissue. Field experiences (senior author) have demonstrated that one batch of inflations manufactured from the same mold and the same type of rubber can dramatically influence the rate of mastitis and milking efficiency because of a change in the physical nature of the rubber with use. Hardness, as an example, will influence the degree of massage and blood circulation at any given vacuum level. As much as 10% difference can be measured in hardness with a Shore A durometer from one batch of rubber to another. Milking efficiency and leucocyte levels usually change when rubber compounding is altered. Research in this area suggests that when narrow bore inflations that measure less than 13/16th inch in diameter become hard and lose tensile qualities because of compounding problems, the teat will not expand sufficiently to permit maximum milk outflow (*7*). These conditions lead to the retention of milk and increased bacterial growth in the udder (*Streptococcus agalactiae*).

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[2]*Provo, Utah.*

Research in Chile investigating non-pulsating milking machines and mastitis suggests that the inflation pinches the teat end and contributes to teat erosions (*9*). Milne (*5*), in evaluating the nature of the streak canal and Firstenberg's rosette in udder defense against disease, stated that any action that is concentrated on the teat end such as the milking machine must take into account the delicate structures of the teat end and their vital role in udder protection since the streak canal of the healthy cow provides an environment continually suppressing microbial growth.

One objective of this study was to investigate the inner surface properties of various inflations after normal use. Size of cavities and pores in the inner surface of the inflation could make cleaning and sanitation difficult to impossible. There is a possibility that inflations after normal use could provide an excellent habitat for bacteria. Dairymen have noted changes in mastitis rate and udder tissue damage following changes in type of rubber inflation which had similar functional properties but with different chemical formulation.

MATERIALS AND METHODS

Teat cup inflations which represented different formulations and use were obtained and washed. The formulations were synthetic rubber and Silastic® (*1*), a silicone rubber. Silastic® is a silicone rubber (TR-55 and TR-70) which is characterized by high tear strength, high modulus, heat stability, nick and abrasion resistance, and good flex life. It imparts no odor and has met wide use in the food-processing industry. Samples were taken from inflations which had not been used for milkings, had been used for 1000 or more milkings and 5000 or more milkings.

Samples of each inflation were taken with a number four cork borer at three locations; middle, 1 inch below teat entry and 1 inch above milk exit. The samples were attached to specimen studs, coated with gold in a CS Mini-Coater sputter apparatus and then viewed with an AMR 1000 A High Resolution scanning electron microscopy (SEM) operating at 20 kv. Pictures were taken at 500, 2000 and 10,000 magnification for all specimens and at higher magnifications for some samples. Permanent records were recorded on the micrographs with a digital data keyboard entry system attached to the AMR SEM.

RESULTS

Rubber: New, no milkings

Figure 1 (A, B, C) represents the inner surface of a rubber inflation that had not been used for milking. There were cracks and openings on the surface which average 0.5 to 1.0 μm in width (*). For each micrograph,

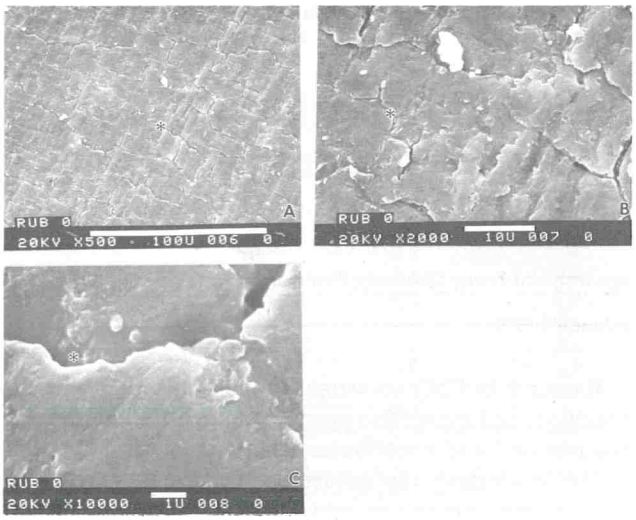

Figure 1. *Magnification (X), Kv, and a micron bar for measurement appear at the bottom of each micrograph. The inner surface of a new rubber inflation at three magnifications (X) is represented. Note the cracks (*) in the surface.*

the magnification was listed (×) and a micron bar is present. There was no marked difference in samples from the three locations of the inflation for all rubber.

Rubber: 1000 + Milkings

Figure 2 (A, B, C) represents the results of examining a rubber inflation that had been used for 1000 to 1200 milkings. The cracks in the inner surface which average 1.0 to 2.2 μm in width (*), have become more prominent when compared to Fig. 1 and apparently harbor bacteria (a). More residue was also present on the surface and in the cracks.

Rubber: 5000 + Milkings

Figure 3 (A, B, C, D) is a series of pictures taken from the inner surface of an inflation used in Chile for 1 year. An additional micrograph was prepared at a lower magnification (49 ×) to show not only cracks in the inner surface but numerous cavities (c) or depressions in the rubber. The cracks were wider (10 to 15 μm in width) in comparison to the previous two figures and caverns were formed (*) harboring what appears to be numerous bacteria (a).

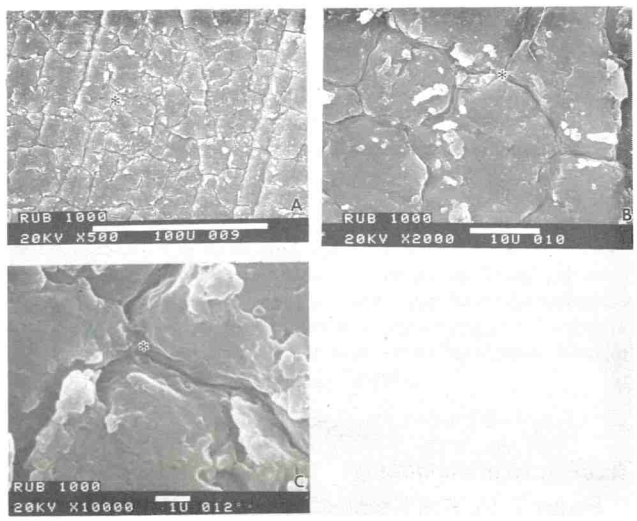

Figure 2. *This is the inner surface of a rubber inflation subjected to 1000 + milkings. The cracks (*) and openings in the surface noted in Figure 1 have expanded in width. Caverns (2C) are forming in the rubber providing potential habitats for bacteria.*

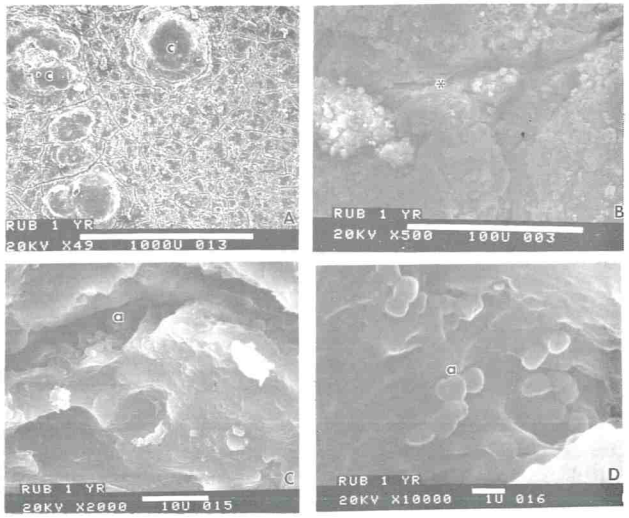

Figure 3. *A rubber inflation subjected to 5000 + milkings or one year's use. Note the cavities (C) in the surface (3A) and the deep caverns (*) in the inflation (3C) which appear to harbor bacteria (A,3D).*

Silastic®: New

Figure 4 (A, B, C) represents a new silicone rubber inflation inner surface. The surface appears smooth and lacks cracks and pores. There was some detritus on the inner surface. The same magnifications and base information are applicable to this series as was used for rubber. There was no marked difference in the three sample locations for silastic® inflations.

Silastic®: 1000 + Milkings

After 1000 + milkings, the inner surface of the inflation appears unchanged in comparison to the new product. There were no bacteria or cracks in the surface [Fig. 5 (A, B, C)].

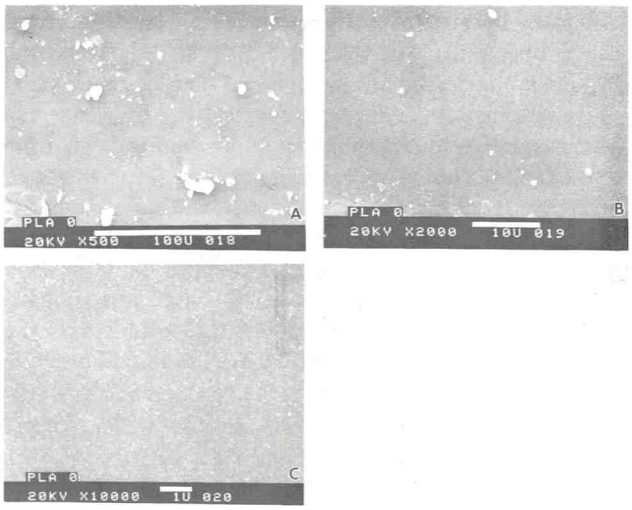

Figure 4. *The inner surface of a new silicone-rubber (Silastic®) inflation is represented at three magnifications (×). The surface is smooth with some debris present. No cracks or cavities in the surface.*

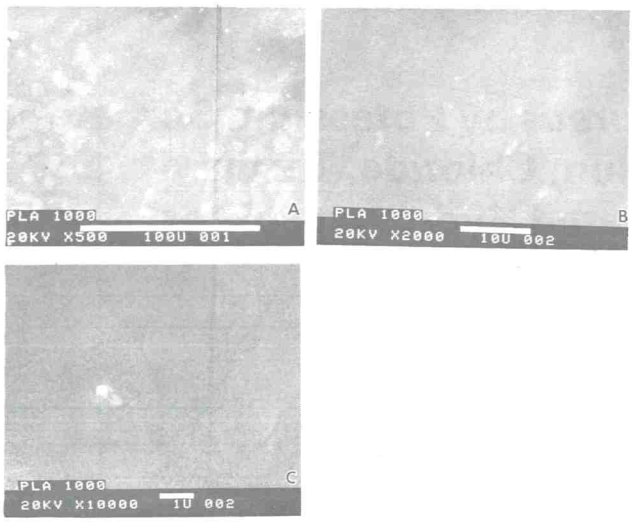

Figure 5. *The Silastic® inflation, after 1000+ milkings, appears smooth. At high magnifications (5C) there are no cracks or breaks in the surface topography.*

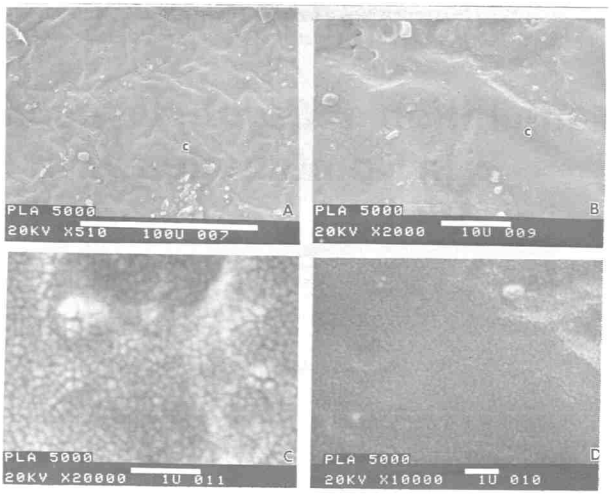

Figure 6. *The silastic® inflation, after 5000+ milkings, starts to show wear. There appear to be folds (6A) in the inner surface with a few pits or cavities (6A, B) present. No bacteria are harbored in the cavities (6C, D).*

Silastic®: 5000 + Milkings

Figure 6 (A, B, C) is a series of pictures representing the inner surface of a silicone rubber product used more than 5000 milkings. A few cavities (C) or depressions appear on the surface but no bacteria were harbored in the depression (Fig. 6, D).

DISCUSSION

Data presented in this study suggest that the inner surface of teat cup inflations can become a reservoir for bacteria. These bacteria could be spread from cow to cow in a dairy herd via the milking machine. Normal sanitation procedure may not be sufficient to eliminate the bacteria from the inflation.

Samples of inflations compounded from the nitrile type of rubber that were used for 1 year on a Chilean dairy herd with a high rate of mastitis were examined. Use of inflations for a year or more is common in many areas of the world. Nitrile rubber is *supposed* to have resistance to mineral and animal fat as well as to aging, often a key advantage over natural gum rubber commonly used in narrow bore inflations measuring less than 13/16 of an inch in diameter.

Natural gum rubber has poor resistance to fat, sunlight, ozone and oxidation. Its use life is comparatively short, when compared to synthetic types of rubber. Results using a scanning electronic microscope (high resolution) showed that silicone rubber has major advantages over both natural and synthetic rubber compounds as a milk contact surface for the manufacture of teat cup inflations.

Field observations of several dairy herds which used silastic® inflations suggest that if the inflation is not designed to permit proper blood circulation in and massage of the teat tissue, the advantages gained through better bacteriological control of the inflation surfaces are lost. Dairymen who used silicone inflations that tended to pinch the teat end during the massage phase and offered little massage on the body of small teats because of a large vacuum chamber in the mouth parts experienced more clinical and subclinical mastitis in their herds. Teat end damage, when comparing types of silicone inflations that reduced teat end pressure and maximized massage on the whole body of the teat, was minimal.

Unpublished research suggests that if silicone inflations are designed to permit proper blood circulation and to minimize pressure differentials across the teat orifice, the incidence of mastitis is reduced in comparison to conventional rubber inflations. The main difference in the inflations studies with SEM was in the porosity of the milk contact surface.

The surface area of milking inflations should be considered as a major source of mastitis-causing bacteria even though the surfaces may appear clean and smooth. Surveys show that most dairymen who use synthetic rubber use them for over 3000 individual cow milkings, and in Latin America, many dairymen use them for a season or longer. Inflation design that requires rubber with a high modulus of elongation requires the use of compounds other than natural gum rubber.

Use of scanning electron microscopy (SEM) should be considered in the evaluation of milk contact surfaces when considering bacteriological problems associated with mastitis control efforts (Fig. 7 A, B, C, D). It was suggested by the senior author in a previous publication (*10*) that some independent agency should draft safety rules for milking machine design to reduce tissue damage and microbial recontamination of the teat orifice. We plan to examine as many inflations as possible with SEM and determine washing effectiveness in the milking parlor.

ACKNOWLEDGMENTS

The authors express their thanks to James Allen who operated the AMR scanning electron microscopy at the Brigham Young University Electron Optics Laboratory for production of the photomicrographs.

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Inhibition of *Staphylococcus aureus* by Potassium Sorbate in Combination with Sodium Chloride, Tertiary Butylhydroquinone, Butylated Hydroxanisole or Ethylenediamine Tetraacetic Acid

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ABSTRACT

The effect of potassium sorbate alone and combined with sodium chloride (NaCl), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) or ethylenediamine tetraacetic acid (EDTA) on growth of two strains of *Staphylococcus aureus* (S-6 and 12600) was studied. The growth studies were made using trypticase soy broth (pH 6.0) at 37 C. Certain combinations of sorbate with NaCl resulted in synergistic inhibition of growth of both strains. The combination of sorbate and TBHQ also resulted in synergistic inhibition of growth of strain 12600, but 25 ppm of TBHQ alone inhibited growth of strain S-6. Certain combinations of sorbate and BHA were synergistic against growth of both strains. Addition of EDTA did not potentiate sorbate's activity against growth of strain S-6 but was synergistic with sorbate against growth of strain 12600.

Several recent studies have demonstrated the antimicrobial effectiveness of phenolic antioxidants in laboratory media (*1,2,3,6,7,8*). Davidson et al. (*2*) reported tertiary butylhydroquinone (TBHQ) to be effective with and without potassium sorbate against growth of *Staphylococcus aureus* in a laboratory medium. Tompkin et al. (*10*) reported that normal levels of butylated hydroxyanisole (BHA) or TBHQ did not enhance the antibotulinal effect of nitrite in a canned meat. The same authors demonstrated that addition of 500 ppm of ethylenediaminetetraacetic acid (EDTA) enhanced botulinal inhibition in pork heart meat. Tompkin et al. (*11*) reported that while EDTA alone is not inhibitory to botulinal outgrowth in pork ham products, addition of EDTA to isoascorbate and nitrite in these products potentiates inhibition of *C. botulinum* in pork ham products.

Pierson et al. (*4*) reported that bacon produced with 0.26% potassium sorbate was inhibitory to *S. aureus* when stored at 13 C. To and Robach (*9*) demonstrated that a 5% potassium sorbate dip inhibited growth of *S. aureus* on fresh chicken. Robach (*5*) demonstrated an interaction between sodium chloride (NaCl) and potassium sorbate against outgrowth of spores of *Clostridium sporogenes* PA 3679.

The purpose of this study was to determine whether potassium sorbate can be combined with BHA, TBHQ, EDTA or NaCl to synergistically inhibit growth of two strains of *S. aureus* in a laboratory medium.

MATERIALS AND METHODS

Cultures

S. aureus strains, FDA S-6 and ATCC 12600, were grown overnight in trypticase soy broth (TSB, BBL) at 37 C.

Inhibition studies

Butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) were obtained from Eastern Chemical Company, Kingsport, Tennessee. BHA and TBHQ were dissolved in 95% ethanol to a final concentration of 2%, filter sterilized (Millipore, 0.22 μ), and stored in a sterile stoppered flask. Ethylenediaminetetraacetic acid (EDTA) was obtained from Sigma Chemical Company, St. Louis, Missouri, as the tetrasodium salt.

Growth studies were done in 250-ml screw capped Erlenmeyer flasks containing 50 ml of sterile TSB (pH 6.0). Appropriate amounts of filter-sterilized BHA, TBHQ, potassium sorbate or EDTA were added to the sterile, cooled medium. Sodium chloride was added to the TSB before autoclaving. The growth flask was inoculated with 1% of an overnight (15-h) culture of the appropriate organism and incubated in a shaker water bath (New Brunswick Scientific, New Brunswick, N. J.) at 37 C and 200 rpm. Samples were withdrawn at selected times, and serial dilutions were made in sterile 99-ml 0.1% peptone dilution blanks before pour plating with trypticase soy agar (BBL). Plates were incubated at 37 C and bacterial colonies counted after 24 h.

RESULTS AND DISCUSSION

The data in Fig. 1 show that sodium chloride combined with 0.2% sorbate resulted in synergistic inhibition of growth of *S. aureus* S-6. Addition of 0.2% sorbate gave an extended lag time and a lower cell count after 36 h of growth. The combination of 5% NaCl plus 0.2% sorbate resulted in very slow growth of strain S-6 through 36 h of 37 C incubation. Figure 2 shows the inhibition of *S. aureus* 12600 by NaCl and sorbate. Addition of 5% or 7% NaCl as well as 0.2% sorbate had only slight effect on growth of strain 12600. When 0.2% sorbate was combined with 5% NaCl, an extended lag period was observed. The combination of 0.2% sorbate and 7% NaCl resulted in an extended lag phase as well as marked inhibition of growth through 36 h at 37 C.

Data in Fig. 3 demonstrate that 25 ppm of TBHQ resulted in no detectable cells of strain S-6 after 24 h with or without 0.2% sorbate. Figure 4 indicates that addition of 25 ppm of TBHQ to the TSB resulted in no growth of strain 12600 and the combination of 0.1% sorbate plus 25 ppm of TBHQ resulted in no detectable cells after 12 h of 37-C incubation.

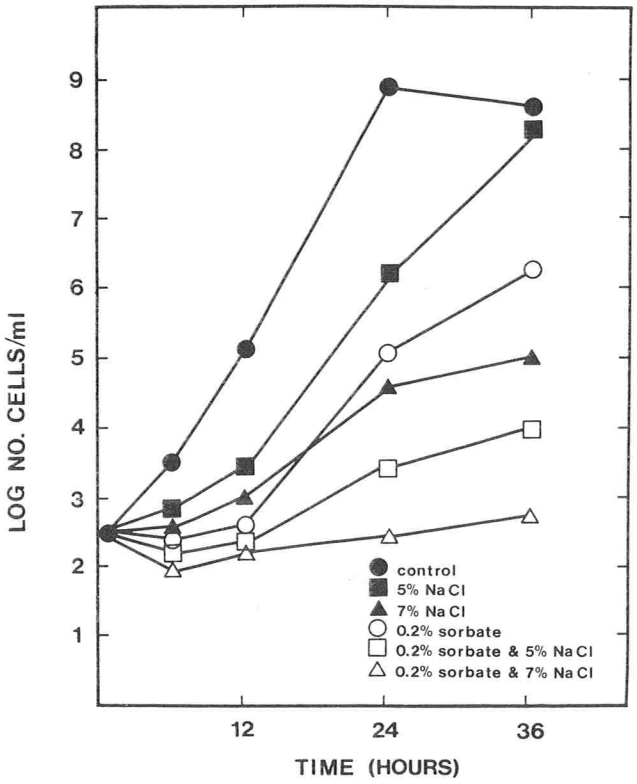

Figure 1. *Effect of sodium chloride and potassium sorbate on growth of* S. aureus *S-6 in trypticase soy broth (pH 6.0) at 37 C.*

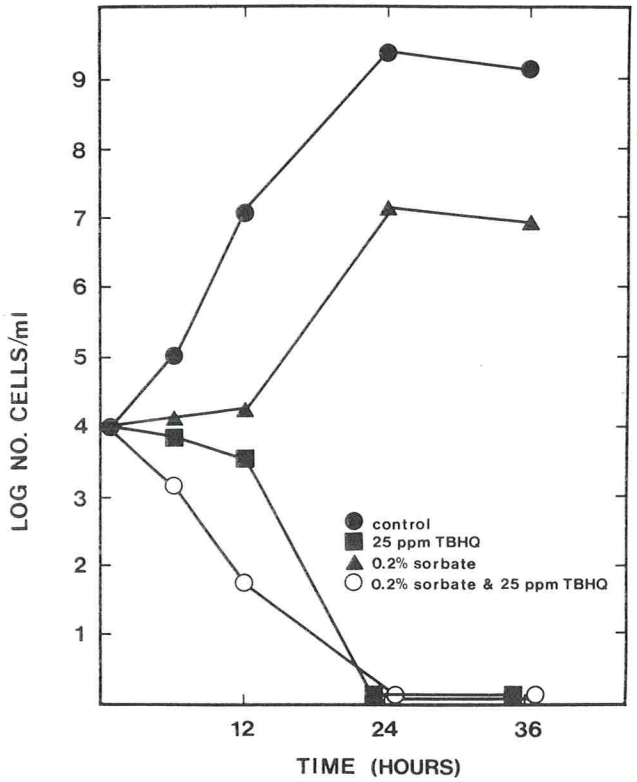

Figure 3. *Effect of TBHQ and potassium sorbate on growth of* S. aureus *S-6 in trypticase soy broth (pH 6.0) at 37 C.*

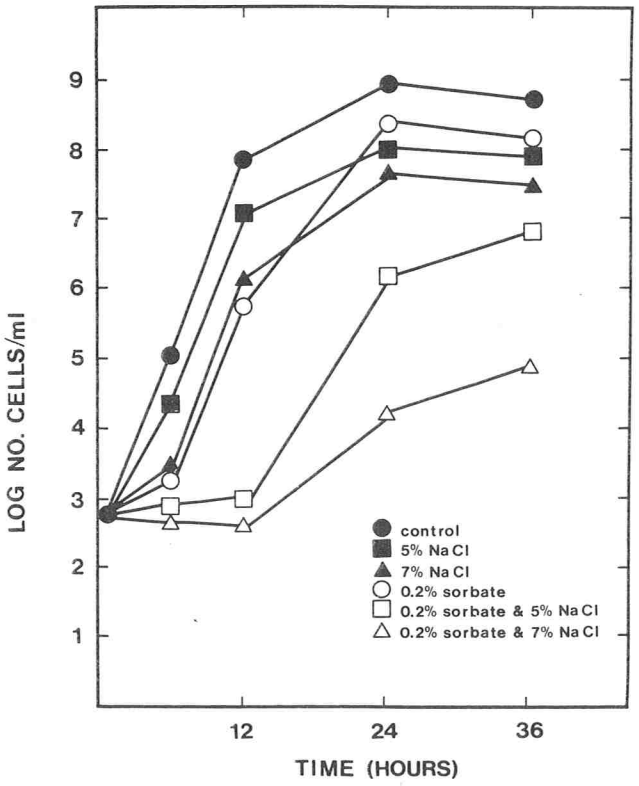

Figure 2. *Effect of sodium chloride and potassium sorbate on growth of* S. aureus *12600 in trypticase soy broth (pH 6.0) at 37 C.*

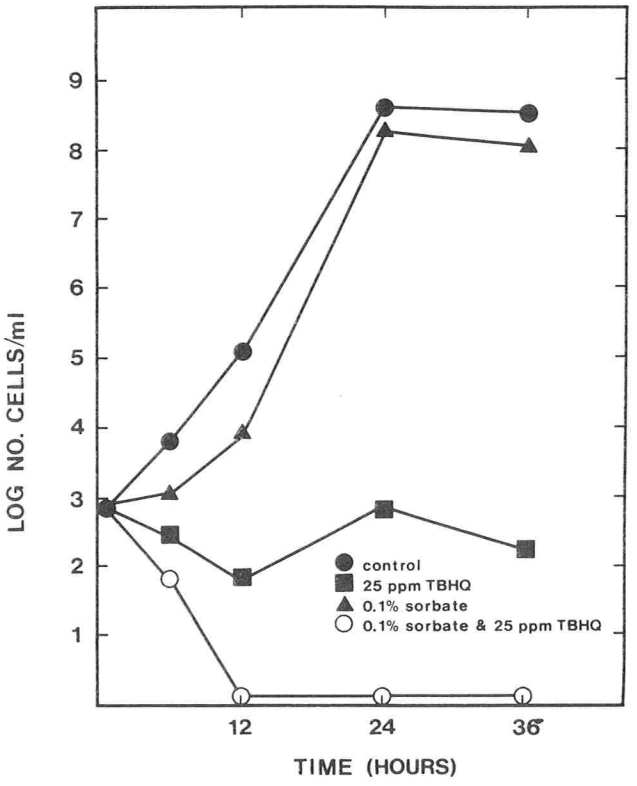

Figure 4. *Effect of TBHQ and potassium sorbate on growth of* S. aureus *12600 in trypticase soy broth (pH 6.0) at 37 C.*

Figure 5 shows that the combination of 0.2% sorbate and 100 ppm of BHA synergistically inhibited growth of strain S-6 in the TSB. Less than a one-log cycle increase was observed through 36 h of incubation. Figure 6 demonstrates the effect of the 0.2% sorbate/100 ppm of BHA combination on the growth of strain 12600. A

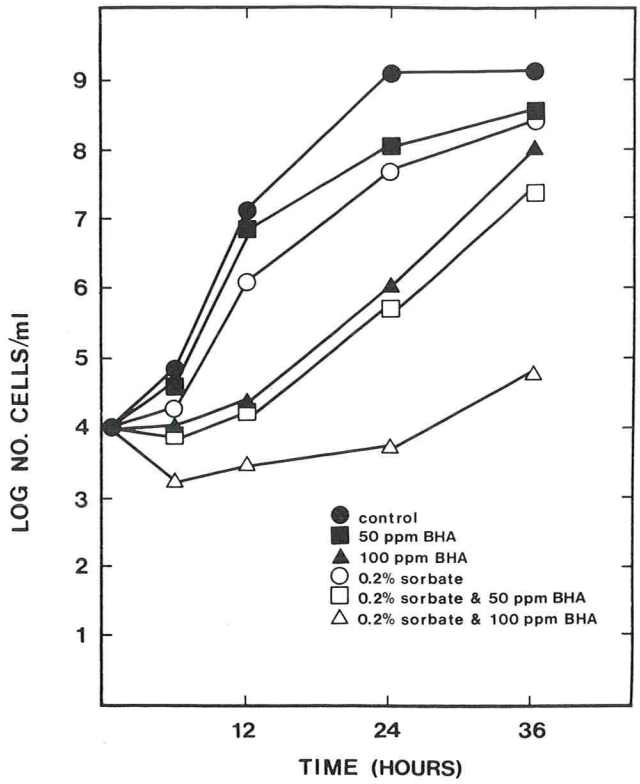

Figure 5. *Effect of BHA and potassium sorbate on growth of* S. aureus *S-6 in trypticase soy broth (pH 6.0) at 37 C.*

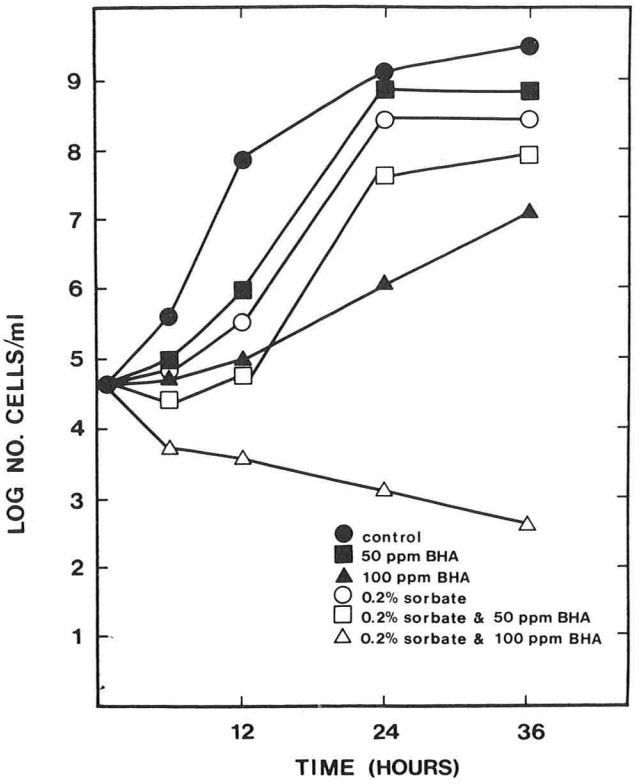

Figure 6. *Effect of BHA and potassium sorbate on growth of* S. aureus *12600 in trypticase soy broth (pH 6.0) at 37 C.*

steady decrease in viable cells was observed during the incubation period.

Figure 7 indicates little interaction between EDTA and sorbate against growth of *S. aureus* S-6. Additive interaction was observable after 36 h of incubation. Figure 8 demonstrates a synergistic interaction against growth of *S. aureus* 12600 when 0.2% sorbate and 50 ppm of EDTA were used in combination.

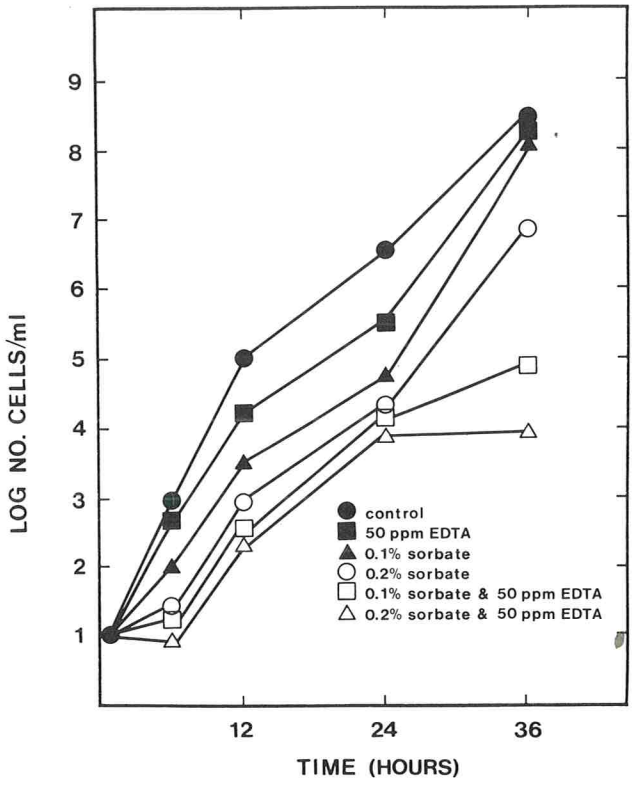

Figure 7. *Effect of EDTA and potassium sorbate on growth of* S. aureus *S-6 in trypticase soy broth (pH 6.0) at 37 C.*

Results obtained in this study and others indicate that the use of antimicrobial agents in combination may result in synergistic inhibition of potential pathogens such as *C. perfringens, C. sporogenes, S. typhimurium* and *S. aureus* (*2,3,5,10,11*).

Results of this study and other studies utilizing laboratory media (*1,2,3,5,6,7,8*) must be looked at carefully and not be extrapolated to food products. Use of fat soluble additives such as TBHQ and BHA in a fat-containing product will result in greatly reduced antimicrobial activity as demonstrated by Klindworth et al. (*3*) and Robach et al. (*7*). However, results of this study and others (*1,2,3,5,6,7,8*) point out the need for more work in this area to study the mechanism of microbial inhibition and the efficacy of these combinations in actual food products.

ACKNOWLEDGMENTS

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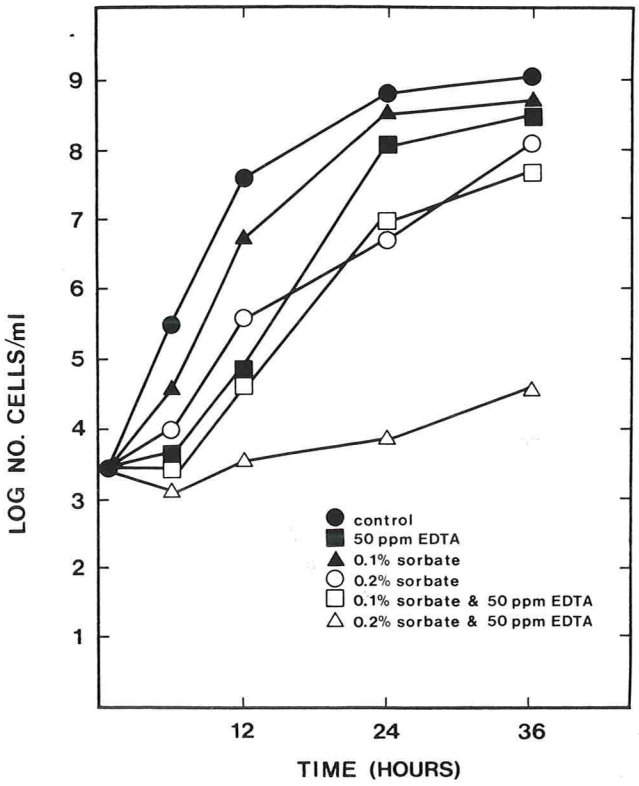

Figure 8. *Effect of EDTA and potassium sorbate on growth of* S. aureus *12600 in trypticase soy broth (pH 6.0) at 37 C.*

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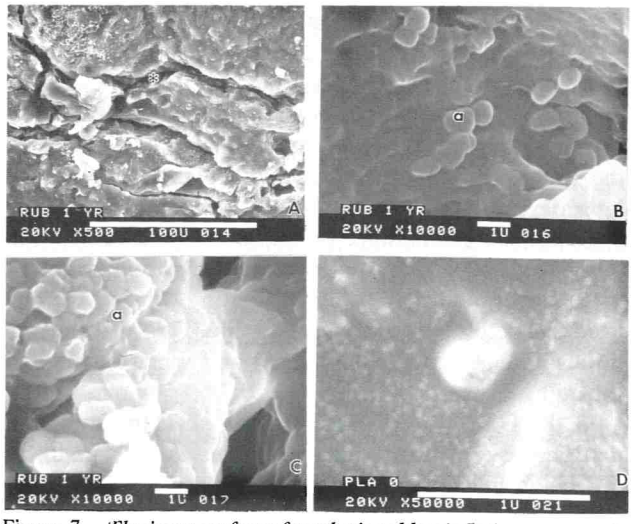

Figure 7. *The inner surface of synthetic rubber inflations appears to be an excellent habitat for bacteria (A: 7B,C) due to use which expands the cracks and openings (*) in the inner surface. Silastic® at high magnification (7D) does not have cracks and openings on the inner surface.*

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Journal of Food Protection Vol. 43, No. 3, Pages 212-225 (March, 1980)

Milk Processed at Ultra-High-Temperatures — A Review[1]

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(Received for publication June 21, 1979)

ABSTRACT

Ultra-high-temperature (UHT) processing today may describe either a sterilization or a pasteurization process. This review primarily covers the sterilization process which involves heating milk at 130 to 150 C for 1 second or more and then packaging it aseptically. The main topics covered are: history, economics, and theoretical basis of UHT processing; common UHT processes and available aseptic packaging systems; microbial, nutritional, biochemical, and physical aspects of UHT milk; and the flavor of UHT milk.

Reviews (*21,41,66,90,99*) and series of articles have extensively scrutinized the topic of ultra-high-temperature (UHT) milk. The most recent of these was an excellent symposium held in Birmingham, Great Britain. Several articles from this symposium (*11,23,28,75,76*) and a comprehensive monograph (*40,72,87*) will be referred to in this review and are highly recommended. While these publications are exhaustive in content, we feel that a review which consolidates and compiles information on all the topics encountered in UHT-milk literature is needed. The last such review was by Burton in 1969 (*21*). Burton's material has been referred to; however, effort has been made to minimize duplication. It has been attempted to make this a selective but by no means a complete review of UHT-milk literature. Wherever relevant, products and systems other than UHT-milk have been included.

Two basically different processes have been labelled UHT. The first, UHT-sterilization, involves heating milk to a high enough temperature for a long enough time to produce a commercially sterile product. The other, UHT-pasteurization, is used prinicipally in North America. Conventional pasteurization involves heating milk at 63 C for 30 min or 71 C for 15 sec. For UHT-pasteurization, milk is heated to a temperature higher than conventional pasteurization long enough to destroy pathogens (*22*). The resulting product is not sterile, although microbiologically it usually keeps better than conventional pasteurized milk. The UHT-pasteurization process was accepted officially in 1965 by the U.S. Public Health Service (*74*). Even in the U.S.A. and Canada, there is an increasing tendency to associate the terminology "UHT-milk" with UHT-sterilized milk (*21,41*). New U.S. Federal Standards of Identity (1978 Pasteurized Milk Ordinance) stipulate that a product labelled "ultra-pasteurized" must have been heated to 137.8 C or above for at least 2 sec (*102*). Detailed "pasteurization" standards for U.S. (*102*) and "sterilization" standards for other countries (*87*) have been established.

Few countries have legal definitions for UHT milk (*87,102*), probably because defining a sterile product is difficult and also because the filling system which affects the bacteriological quality of the milk cannot be controlled by the "definite temperature for a definite time" description (*21*).

In this paper, other than some specific references, the term "UHT-milk" will refer to milk that has been heated to at least 130 C for not less than 1 sec and then aseptically packaged. In general, UHT-milk processing involves temperatures of 130 to 150 C and holding times of 1 sec or more (*21*); 2-8 sec is the generally accepted holding time (*41*). Burton (*23*) recently suggested that below 135 C, the sterilizing process was unlikely to be sufficient for a commercial process unless the holding time was made undesirably long; conversely at above 150 C, the required holding time would probably be too short to be maintained effectively.

Burton (*21*) and Westhoff (*102*) summarized development of modern UHT-milk. Jonas Nielsen had pioneered the first recorded UHT processing plant by 1913 and later developed an aseptic canning system. The modern trend toward UHT, however, started in the late 1940s as plants started using higher processing temperatures, with correspondingly shorter holding times, to give products better bacteriological quality. This also resulted in less change in the color, flavor and nutritive value of the milk.

In the U.S., "fresh-tasting" long shelf-life milk was produced by sterilizing milk in bottles as early as in the 1940s and in cans in the 1950s (*41*). Because these milks were sterilized inside containers, their flavors were not as good as the flavor of today's pasteurized milk. In 1958, a rectangular polyethylene-coated paperboard container, with an aluminum foil laminate barrier, was introduced by Tetra Pak in Switzerland. After Switzerland, aseptically packaged milk entered Germany, Italy, France, United Kingdom, South Africa and other countries including Canada. In many European countries, UHT-milk is making strong inroads into fluid milk markets, e.g. as much as an estimated 50% in Italy and Germany in 1976 (*75*).

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ECONOMICS

The economics of UHT-milk are dependent on processing costs and distribution costs. Neitzke in 1965 (*66*) compared the costs of four sterilization processes with the costs of pasteurized milk in Germany. Processing costs for UHT-milk (Uperization process) were the highest, and for pasteurized milk the lowest; this was mainly because disposable cartons were used to package the Uperized milk, whereas returnable bottles were used for other milks. Costs for cartons of UHT-milk were also compared to costs for bottles of pasteurized milk in the U.K. (*78*); UHT-milk was more expensive to produce; however, if both processes had used cartons, then presumably the processing costs for the milks would have been nearly equal. Since its introduction in 1965, the price of UHT-milk in the U.K. has remained 23 to 27% above the price of regular pasteurized milk (*28*). Bene (*13*) observed (Table 1) that in Norway, UHT-milk was more expensive to process and package than pasteurized milk. Distribution costs and other factors might equalize the processing costs.

Currently, UHT-milk appears to be competitive to pasteurized milk. In 1976, the average price of UHT-milk in Germany was 1.8% lower than that of pasteurized liquid milk (*73*). A 2% UHT-milk which was introduced in Canada on September 17, 1975, had in 2 years captured 20% of the fluid milk market in the areas it served (*7*). The UHT-milk sells for about 1 cent premium per liter (*6*). Exports account for 20% of the sales of the Canadian UHT-milk (*7*). According to a study by the Manitoba Department of Agriculture in Canada, processing and distribution costs for UHT-milk are between 65 and 85% of those for pasteurized milk (*7*). Based on these studies, it appears that marketing UHT-milk in the U.S. might be economically viable.

Many workers (*41,77,78*) contemplate that if UHT-milk is widely accepted in the U.S., it will change the entire structure of the dairy industry. Production schedules would probably require only 5-day weeks instead of 6 or 7 days. In periods of excess milk production, instead of diverting the excess to marginal products such as non-fat dry milk or cheese, UHT-milk could be stockpiled. Processing plants would be moved away from cities to the milk-producing areas. Labor would be greatly reduced as the operation of UHT units is highly automated. The need for expensive refrigerated storage would be drastically reduced, and inexpensive warehouses could be used.

Home delivery would cease completely. Refrigerated display cabinets, now crowded, could be used to display items other than milk because milk would be displayed at room temperature. Milk could be delivered to the supermarkets once a week or twice a month. It would be shipped in unrefrigerated flat-top containers by pallet load rather than in wood, wire or plastic cases. Distance would no longer limit shipments; UHT-milk could be shipped almost anywhere a dairy could find a buyer.

Quality control tests would change from microbial quality to other factors, such as off-flavor, milk separation, and gel formation. Consumers would purchase dozens of quarts of milk, or milk products, at one time and store them in basements. Milk would be refrigerated only a few hours before consumption. The costs of processing and packaging the milk would increase, but the distribution costs would be reduced drastically.

TABLE 1. *The costs of UHT milk compared with pasteurized milk in Norway.*[a]

Item	UHT milk[b] (Cents/liter unit)	Pasteurized milk[c] (Cents/liter unit)
Packaging material	4.48	2.81
Equipment	0.63	0.29
Labor	0.35	0.20
Energy	0.42	0.07
TOTAL	5.88	3.37

[a]Condensed from Bene, 1974 (*13*). Based on production of one million liter units/year and an exchange rate of 1 Norwegian Kroner = 0.1888 U.S. dollar.
[b]Packaged in Tetra Brik cartons.
[c]Packaged in Pure Pak cartons.

THEORETICAL BASIS FOR UHT-PROCESSING

Microorganisms are destroyed by heat when the microbial proteins coagulate and enzymes required for their metabolism are inactivated (*35*). Heat treatment necessary to kill microorganisms or their spores varies with the kinds of organisms, their state, and the environment during heating. A term used to express the heat resistance of microorganisms is "thermal death time" (TDT), defined as the time at a certain temperature necessary to kill all of the organisms (or spores) under specified conditions. Processing times and temperatures are based on a TDT curve for whatever organism the process is designed to destroy (*67*). The TDT curve is obtained by plotting the logarithm of TDT versus temperature (*35*).

In food processing operations, the equation normally used to determine the processing time is called the F_0 or the sterilization equation (*41*):

$$\log \frac{F_0}{t} = \frac{T - 250}{z}$$

where, T = the processing temperature in degress Fahrenheit (F)

t = the holding time in minutes

z = slope of the TDT curve or the degrees F required for the TDT curve to traverse one log cycle

F_0 = the sterilization value or number of minutes required to destroy all organisms at 250 F.

From the sterilization equation, if three values are known, the fourth can be calculated. For convenience, alignment charts (nomographs) also are available to compute one value when the other three are known (*39*).

In the 110-125 C range, the rate of spore destruction for *Bacillus stearothermophilus* increases about 11 times for each 10-C rise in temperature (*34*). This relationship

also probably holds true beyond the 110-125 C range. Other *Bacillus* spores such as those of *Bacillus subtilis* are more sensitive, and their rate of destruction increases 30 times for every 10-C increase in temperature (*41*). Maillard browning, an important chemical reaction during UHT-processing, occurs when proteins interact with reducing sugars. This results in off-flavors and discoloration. Burton (*18*) determined that in the range of 95 to 120 C, the rate of browning increases about three times for each 10-C rise in temperature. He plotted a curve for the ratio of bactericidal (sporicidal) effect to browning effect versus temperature, as shown in Fig. 1 (*22*). The temperature quotient (Q_{10}), or the rate of increase of spore destruction for every 10-C increase in temperature, is designated as 20, (average for *B. subtilis* = 30 and *B. stearothermophilus* = 11) and that for browning is 3. The ratio does not change much until the temperature reaches about 135 C. Above 135 C, the bactericidal effect increases rapidly in relation to browning. At 150 C, the bactericidal effect is about 5000 times greater. Therefore, if milk is treated in the UHT range, 135-150 C, for a few seconds, it is possible to obtain a product virtually free of spores and with much less browning than milk from conventional in-can sterilization. The color of this product is similar to that of HTST (high temperature-short time) milk. Recently Woods (*109*) used *Clostridium botulinum* to make a similar comparison between sterilization rate and rate of chemical changes at UHT temperatures. UHT milk sterilization is based on these basic principles.

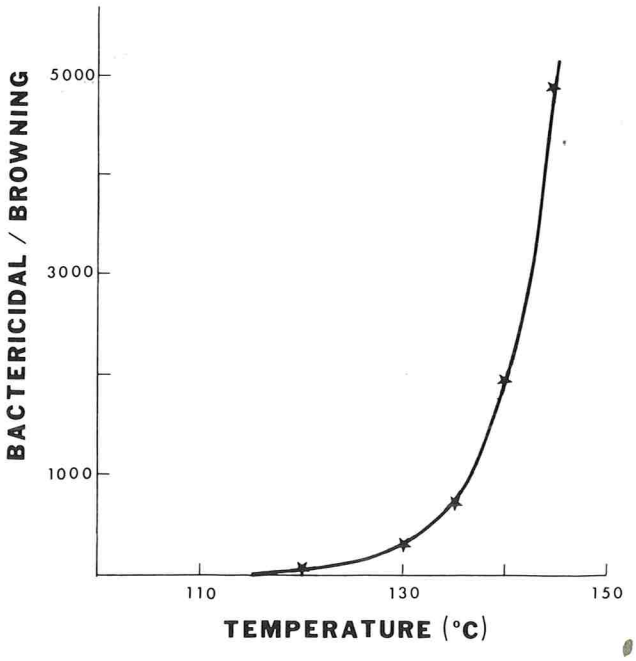


Figure 1. *The effect of increasing temperature on the ratio of bactericidal effect to browning during sterilization. Q_{10} for spore destruction = 20; Q_{10} for browning = 3. Ratio = 1 at 100 C (22).*

PROCESS DESCRIPTION

UHT-sterilization

UHT processes can be classified as either directly heated or indirectly heated, according to the kind of heat exchangers used. In processes using indirect heat, milk is heated through a heat-conducting barrier, usually stainless steel, which separates the heating agent from the milk. The heating surface may be either a corrugated plate, as in many HTST pasteurizers, or a tube with a relatively small diameter. Alternatively, the heat exchanger may be of the scraped-surface type, in which the milk flows through a large cylinder. The cylinder is heated externally by steam, and the inside wall of the cylinder is scraped by continuously rotating blades. The scraped-surface system is rarely used for milk but is suitable for viscous products in which it is difficult to induce turbulence (*21*).

In processes using direct heat, milk is mixed with saturated steam under pressure and heated rapidly as the steam condenses (*21*). Steam may be injected into the milk, or the milk may be sprayed into an atmosphere of steam. The heated milk is injected through an orifice into a vacuum vessel where water added to milk as condensate is removed, thereby cooling the milk. In one recent modification of the milk-into-steam technique, a free-falling-film UHT system (*5,52,103*) was developed. Here the product falls through a steam chamber as a thin, laminar, free-falling film. The final product is sterile and is reportedly similar in flavor to pasteurized milk.

In processes using direct heat, there also are indirectly-heated stages, with plates and tubes for preheating, regeneration and cooling. Conversely, equipment for indirect heat may sometimes include vacuum flash vessels to remove gas and vapor (*21*). Recently, complex UHT processes have been simplified by minimizing the number of intermediate steps (*11*). A special requirement for equipment using direct heat is that the steam be culinary and contain no toxic compounds (*21*).

UHT processes available have been summarized by Burton (*21*), Hsu (*41*) and Ashton (*9*). These have been combined in Table 2. In addition to these, Burton (*23*) stated that in one commercial system available now, the heating is from electric resistance (electric heaters), and in another heat is generated by friction, by passing a thin film of milk across the face of a high-speed rotor. Milk has also been sterilized by microwave energy, although not commercially.

Aseptic packaging

An aseptic filling process to avoid bacterial contamination after sterilization treatment is necessary for UHT-sterilized milk. Any aseptic filling installation must sterilize the container, fill it, and then seal it without contamination. Thus aseptic filling must be a single operation to carry out these functions.

The existing fillers can be classified according to either the kind of container filled, i.e. cans, cartons or bottles, or the differences in the basic principles used in the aseptic packaging machinery. The major operational

aseptic packaging systems for UHT dairy products are (*41*):

(*a*) *Aseptic Tetra Pak.* The Aseptic Tetra Pak was the first aseptic packaging machine used to package UHT milk in paper containers and was derived from the conventional Tetra Pak filler. Major problems in this system are the sterilization of paper inside the containers just before filling, keeping the condition aseptic during filling and making an aseptic seal after filling.

Both physical and chemical means have been used to sterilize the paper. Physical means include heat treatment, ultraviolet radiation, and high frequency electric fields. Chemical means include treatment with ethylene oxide, available chlorine or hydrogen peroxide. Hydrogen peroxide, combined with a subsequent high temperature treatment of the Tetra Pak paper, has become the main technique today. The Tetra Pak carton is formed from a polyethylene-coated paper strip and filled on the same machine. As the paper leaves the reel, a strip of polyethylene is added to reinforce the longitudinal seam. The strip of paper passes through a bath of dilute hydrogen peroxide; the excess is removed by squeezing to leave a thin film. After the paper has been formed and heat-sealed into a vertical tube, it passes an electric heating element totally enclosed by the tube. The heating element raises the inside surface temperature of the paper to 200-250 C, decomposing the hydrogen peroxide to steam and nascent oxygen; the nascent oxygen sterilizes the surface. A thermally insulated supply tube conveys the milk down through the heating element into the paper tube, which is then cut and heat-sealed transversely to give tetrahedron-shaped cartons.

(*b*) *Aseptic Tetra Brik.* The Aseptic Tetra Brik was designed specially for aseptic packaging. The Tetra Brik process is similar to the Tetra Pak process and consists of three steps: from a reel, plastic-coated paper is shaped into a tube; filling is continuous into this tube and rectangular packs are produced after the tubes are sealed transversely below the surface of the product. Commercial Tetra Brik carton board is laminated (from inside out) with two layers polyethylene, a layer of aluminum foil, polyethylene, two layers of paper, printing ink and a layer of polyethylene (*94*). Some unique characteristics of the Tetra Brik are: the shape of the container is rectangular, application of the sterilant (hydrogen peroxide) has been simplified - the peroxide bath itself is at 80 C and instead of squeezing rollers a new device known as "air-knives" removes excess hydrogen peroxide by means of an air-jet.

(*c*) *Aseptic Pure Pak.* The aseptic Pure Pak system was developed by the Ex-Cell-O Corporation of Detroit. The Pure-Pak system differs from other aseptic systems in that it relies only on chemical sterilization of the containers rather than the combination of chemical sterilization and heat; also Pure Pak operations are carried out in a "sterile" environment rather than a "sterilizing" environment. The former involves sterilizing the packaging system at the beginning of a day and then maintaining a positive pressure of sterile air within the system for the rest of the day, whereas the latter involves sterilizing the entire environment of the package within the packaging system by heat or other means throughout the period of operation.

TABLE 2. *Commercial UHT processing equipment.*

Category	Name of process	Developed and/or manufactured by
INDIRECT HEATING		
Tubular type	Sterideal *and* Mini-Sterideal	Stork of Holland
	CTA system	CP Division, St. Regis, U.S.A.
	Spiratherm and Unitherm	Cherry-Burrell of U.S.A.
	Roswell	Roswell of U.S.A.
	Mallorizer	Mallory of U.S.A.
	Graves-Stambaugh	Graves-Stambaugh of U.S.A.
	Schmidt	Schmidt
	Gerbig	Gerbig of Germany
Plate type	Ultramatic	APV of England
	Dual-purpose system	Alfa-Laval De-Laval Group of Sweden
	Sterilpak (Sordi-Lodi)	M. Sordi of Italy
	Ahlborn	Ed. Ahlborn of Germany
Scraped surface	Swept Surface heater	CP Division, St. Regis of U.S.A.
	Votator Scraped Surface heater	Votator Division, Chemetron Corp., U.S.A.
	Therutator heater	Cherry-Burrell Corp., U.S.A.
DIRECT HEATING		
Steam-into-milk (injection)	Vacu-Therm Instant Sterilizer (VTIS)	Alfa-Laval/De-Laval Group of Sweden
	Uperization	Alpura Ltd. & Sulzer Bros. of Switzerland & APV of England
	Aro-Vac system (No-Bac Aro-Vac)	Cherry-Burrell Corp. of U.S.A.
	Grindrod	Smith, Kline and French of U.S.A.
Milk-into-steam (infusion)	Laguilharre	Ets. Laguilharre of France
	Thermovac (Thimonnier)	Breil & Martel of France
	Palarisator	Paasch & Silkeborg of Denmark
	Ultra Therm	Creamery Package Division, St. Regis of U.S.A.
	Free-falling-film	DaSi Industries of U. of Maryland, U.S.A.

(*d*) *Dole Aseptic Canning System.* This process was patented by the James Dole Engineering Co. of Nevada. The original system consisted of UHT sterilization and aseptic canning linked together to form a complete system for heat-sensitive foods. For UHT-milk, only the aseptic canning part is used with one of the UHT-milk sterilizers. The packaging system consists of five major sections: can sterilizer, cover sterilizer, filling chamber, closing and sealing machine and the controls. Superheated steam at 260 C is used to sterilize the cans and covers.

(*e*) *Anderson Formseal.* This machine was developed by the Anderson Brothers Manufacturing Company of Rockford, Illinois, mainly to package coffee cream or whiteners in small half-ounce plastic containers formed from heat-treated polystyrene sheets. Pressure is applied to shape the plastic into cups before they are filled with the sterilized product. The covers are treated both chemically and with ultra-violet light before being sealed to the cups.

(*f*) *Dole System for glass jars and bottles.* This was designed by the James Dole Engineering Co. and the Glass Manufacturers Institute.

(*g*) *GEM-NIRD Aseptic Bottling Machine.* This was designed by the National Institute for Research in Dairying at Reading and the Graham-Enock Manufacturing Co. Ltd., London, England.

MICROBIOLOGY

The shelf life of a UHT-product depends mainly on two factors: the sterilizing effect of the UHT-equipment, and the aseptic condition of the packaging. To assure quality, the product should be sampled at two stages: after the UHT heating unit and after the finished product is packaged (*41*).

A recent Russian survey (*55*) determined that the three main causes of microbial contamination in UHT-milk plants are lowering of sterilization temperature, inefficiency and carelessness in sterilizing the equipment and failure to ensure asepsis during packaging. A complete quality assurance program for UHT-milk was outlined by Roberts (*76*).

As complete sterility is approached, the number of microorganisms that survive is extremely small. Such a small number can be detected only when a huge volume of sample is examined. To make the analysis more practical, a pre-incubation technique is usually used. This technique was developed to increase the number of viable bacteria, or spores, if any, by pre-incubating the test samples at a suitable temperature for a suitable time. The conditions usually recommended are 32, 37, or 55 C for 5, 7 or 10 days. Under these conditions, if the product sours or coagulates, the presence of viable spores or organisms is established. Further, bacteriological analysis of all the samples can be made much more easily after this treatment. Complete asepsis must be provided when a UHT-product is analyzed because a single contaminant could confuse the entire situation.

A Finnish company (*60*) has recently developed a device to automatically control the quality of UHT-milk. This device operates on the basis of electrical registration of changes in hydrodynamic properties (e.g. viscosity) due to microbial activity. It checks each package separately and automatically rejects faulty packages which have microorganisms in the milk. Another potentially useful new tool is a lethality computer developed in the U.K. (*86*). The instrument computes F values continuously throughout a heat sterilization process from a temperature signal derived from a thermistor probe. The displayed F value is claimed to be accurate within 3%.

Wajid and Kalra (*100*) have described the use of an enzyme in increasing the shelf-life of a sterile milk. The shelf-life of sterilized milk inoculated with *B. subtilis* or *B. stearothermophilus* (100 spores/ml) and heat-treated at 109 C for 5 min was increased from 3-7 days to 60 days in the presence of 100 Reading Units (RU) of nisin/ml. A RU is a 25 parts per billion solution of the enzyme. Mahmoud et al. (*57*) obtained similar results with *B. subtilis* in pasteurized milk but found that 100-1000 RU of nisin/ml did not extend the shelf-life of milk inoculated with *Escherichia coli, Enterobacter aerogenes* or *Pseudomonas fluorescens.*

Cunliffe et al. (*27*) reported that pasteurization could not inactivate foot-and-mouth disease (FMD) virus in milk but UHT (148 C for 3 sec) could. UHT treatment of dairy products has been suggested as a means of eliminating regional or international distribution of infectious FMD virus.

NUTRITION

The nutritive value of UHT-milk can be reduced at two stages, either during UHT treatment or during storage. Nutritive value usually is lost because of changes in the chemical structures of the nutrients.

The effect of UHT processing conditions differs for the various nutrients of milk. The nutritive values of some components such as the fat, fat-soluble vitamins, carbohydrates and minerals are essentially unaffected, whereas values of other components such as the water-soluble vitamins and proteins are adversely affected (*72*). Proteins, especially the whey proteins, are denatured.

During storage, the main factors affecting nutrients are temperature, light and oxygen. The main nutritional changes that occur in milk during storage are associated with the vitamin components. Proteins are affected but not to any appreciable extent (*72*).

Vitamins

Burton (*21*), van Eekelen and Heijne (*99*) and Porter and Thompson (*72*) have reviewed in detail the effects of high heat treatment and prolonged storage on stability of vitamins in milk. They have observed that in general, vitamins are more stable under UHT processing conditions than under pasteurization or other "low temperature" heat treatments (*21,72,99*).

Vitamins A, D, E and carotene are little affected by high heat treatments used during milk sterilization (*21,72,99*). Vitamin A and carotene losses up to 35% have been observed on prolonged sterilization (*99*). Workers have reported a loss of up to 6% of vitamin D (*99*) and less than 10% of vitamin E (*21*) during UHT processing. Negligible loss of vitamins A and E was observed in UHT-milks stored at ambient temperature for 180 days (*21*).

Pantothenic acid, nicotinic acid and biotin are not affected by UHT treatments (*21,72,99*) and storage (*21*) of milk. Considerable variation has been observed in the losses of the other water-soluble vitamins during high heat treatments and subsequent storage. Losses reported vary from none up to 100% for some of the vitamins. For thiamine, losses greater than 20% have been observed during different UHT treatments and up to 50% with prolonged in-bottle sterilization. Riboflavin, which is stable to heat, is susceptible to the action of light (*99*). Less than 10% losses were observed in riboflavin during sterilization (*21*), but losses up to 60% were observed during a 3-month storage period (*99*). Results for vitamin B_6 vary considerably in the literature (*21,99*). Van Eekelen and Heijne (*99*) suggested that these variations could be caused by differences in the original amounts of the vitamins in milk (the higher the initial concentrations of the vitamins in milk, the greater the losses), the assay technique and the storage time of the milk.

Losses of ascorbic acid, folic acid and vitamin B_{12} are interrelated. Ascorbic acid is heat-stable but in the presence of oxygen is converted to heat labile dehydroascorbic acid. Because most of the results published have not distinguished between the two forms of the vitamin, UHT heat treatment losses of 0 to 30% have been reported. Ascorbic acid surviving heat treatment may be lost in storage (*21*). If the amount of oxygen dissolved in milk is limited, ascorbic acid losses are minimal (*21,72*). Thus heat treatment plays a minor role in destroying ascorbic acid in milk; oxygen availability is the critical element. Low oxygen levels are achieved by either the use of a deaerator before heating or evaporative cooling after processing (*72*).

Folic acid appears to be stable on processing and storage as long as reduced ascorbic acid is present in milk. After ascorbic acid is eliminated, folic acid is lost rapidly (*21,72*). During in-bottle sterilization, the greater part of vitamin B_{12} is lost (*99*); however, under UHT conditions losses of only 30% have been recorded (*21*). During storage, losses of 60% have been reported after 90 days (*99*) and 180 days (*21*). The smallest losses were found for a plant using a deaeration unit before the UHT state of processing (*21*).

Minerals

Hansen and Melo (*37*) found that free calcium was reduced significantly in milk processed at 143 C for 8 to 10 sec. The free calcium could have been transformed into a colloidal form or could have precipitated along with other milk constituents. Experiments with rats have shown no change in the availability of the calcium of milk after indirect UHT processing (*21*). Pelet and Donath (*70*) used eight newborn infants to test the effect of Uperization of "humanized" cow's milk. "Humanized" cow's milk is milk that has been modified to conform closely in composition to human milk (*33*). Uperization did not affect nitrogen balance and phosphorus retention; however, calcium and potassium retention was higher in infants receiving Uperized milk than in those receiving pasteurized milk (*70*).

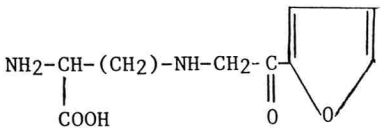
Protein

Severe heat treatment causes considerable denaturation (up to 80%) of the serum proteins of milk, especially β-lactoglobulin (*21*). UHT-milk heated directly had smaller amounts of serum protein denaturation than indirectly heated milk. β-Lactoglobulin, on denaturation, apparently complexes with casein. UHT processing increases the size of the casein aggregates and changes their composition (*21*). White and Sweetsur (*104*) did not observe any significant changes in the rate of heat-induced (110-140 C) aggregation of casein by removal of serum proteins or colloidal calcium phosphate or by prevention of K-casein/β-lactoglobulin interaction. During storage of UHT-milk, the α- and β-casein electrophoretic peaks become broader and less well-defined, with increases in non-casein and non-protein nitrogen (*21*). Increasing length of storage increases proportion of the casein aggregates; however, these is no corresponding increase in size of aggregates (*24*).

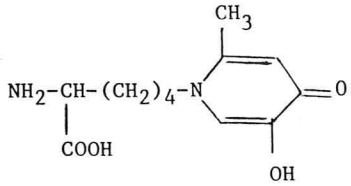
An increase in sugar content has been shown within the α-casein band, indicating a preliminary stage of the amino acid-sugar Maillard reaction (*21*). Isotope studies (*98*) with lactose and milk proteins has established that when heated together in model systems at 143.5 C for 10 sec, casein micelles incorporated 5 to 6 times the amount of lactose as α-lactalbumin and 8 to 12 times as much β-lactoglobulin; the lactose appears to be covalently bonded to the protein fractions.

Lysine levels in milk are reduced by milk sterilization; however, methionine and tryptophan which also are heat-labile were not much affected (*99*). On the other hand, Aboshama and Hansen (*1*) observed a 34% loss of cysteine and cystine (as cysteic acid), and methionine in skim milk during UHT processing versus only 5-10% during pasteurization. No significant changes have been observed for the other amino acids either during processing or storage of the UHT milk (*99*).

Two new basic amino acids have been detected by Finot et al. (*30*) in the acid hydrolysates of scorched roller-dried milk powder. Apparently furosine and pyridosine, which are the trivial names given to these new amino acids by the authors, are formed from ε-N-(1-deoxy-D-fructosyl)-L-lysine (or fructose lysine) by the loss of three molecules of water. The structures of these compounds have been determined and their possible mechanism of formation has been postulated. The structures of these amino acids follow:



Furosine



Pyridosine

Animal feeding studies of UHT-milk have shown that, in general, the biological value, protein efficiency ratio and digestibility coefficient are not affected by UHT sterilization (*21,72*). Some studies, on the other hand, have shown that with denaturation of serum proteins, there is a proportionate decrease in the growth-supporting value of the milk protein for test animals. It was concluded that denaturation is not a significant factor in human infant nutrition (*21,72*). However, a recent study (*7*) changes this hypothesis. In a feeding test involving 400 newborn infants in Holland, 200 children drinking UHT-milk gained an average of 7 g more weight per day than the 200 drinking pasteurized milk. In addition, the normal weight loss immediately after birth was regained sooner by the UHT-fed infants. The UHT-milk also caused fewer digestive problems.

Unsaturated fatty acids

The loss of polyunsaturated fatty acids, in which milk is already poor, may be important. Heating milk at 130 C for 20 sec causes a loss of 34% linoleic, 13% linolenic, and 7% arachidonic acid (*99*).

BIOCHEMICAL AND PHYSICAL ASPECTS

Enzymes

Phosphatase activity is always zero after milk has been sterilized but may be reactivated after prolonged storage. The higher the storage temperature and the longer the storage time of the milk, the higher the degree of reactivation of the enzyme. According to some workers (*41*), this reactivation phenomenon may be an artifact caused by liberation of sulfhydryl groups which also react with the phosphatase test reagent and thus yield a positive test. Other workers (*21*) have established that the reactivated enzyme is identical in chemical form to the original enzyme and this reactivation is enhanced by free SH groups but retarded by oxygen and lower storage temperatures.

Peroxidases are almost always destroyed and proteases are usually destroyed at UHT sterilization temperatures. No reactivation of peroxidase has been observed under any storage condition. Some researchers (*2,21*) have suggested that proteases may be reactivated on storage. Kielwein (*50*) has suggested that UHT processing may not ensure complete inactivation of *Pseudomonas fluorescens* proteases. Adams et al. (*2*) found that 70-90% of raw milk analyzed contained psychrotrophs which produced proteases that survived 149 C for 10 sec. These authors suggest that thermal input needed to destroy heat-resistant proteases would damage the milk severely. However, West et al. (*101*) suggested an effective method of inactivating proteases; when UHT-treated (149 C for 4.5 sec) skim milk was maintained at 55 C for 1 h, an average of 88.5% of the heat-resistant proteases were destroyed. Barach et al. (*12*) determined that bacterial protease activity in buffered solutions was reduced 90% during 10 min at 55 C. In either raw or sterile milk, however, the extent of inactivation was less, e.g. 70% during 60 min at 55 C. The rate of inactivation did not depend on protease concentration.

Kiermeier and Doruk (*51*) have demonstrated that β-glucuronidase in milk is completely inactivated by pasteurization (63 C for 30 min or 72 C for 15 sec) but not always fully by UHT processing. The heat liability (up to a maximum of 100 C) of lipase (*46,84*), catalase (*46,84*) and adolase, α-amylase, β-amylase, lysozyme and ribonuclease (*84*) also has been reviewed.

Greenbank and Pallansch (*36*) found that heating whole milk to 90 C for 15 sec inactivated xanthine oxidase. Yet, when the same milk was condensed to 50% total solids and homogenized at 4,500 psi, some reactivation was noted; this reactivation was not observed in powders from milks heated 93 C.

Sedimentation, gelation and burn-on

The intensive heat from the heating agent during UHT processing usually causes denaturation of the milk proteins or precipitation of the salts in milk; this causes sedimentation. The higher the sterilizing temperature, the more the sedimentation. Maximum sedimentation in UHT processing at 140, 145 and 150 C occurs with a 4-sec holding time (*21,41*). In general, sedimentation in UHT-milk is not a serious problem; in a recent study the average sediment of 77 trials was only 0.5 μl/cc (*41*). Calcium balance and addition of salts affect sedimentation. Adding sodium citrate or bicarbonate inhibits sediment formation, but calcium promotes sedimentation (*41*). Biryukova et al. (*15*) established that adding 0.025-0.1% sodium citrate or disodium phosphate increased the heat stability of milk against sedimentation by 50 to 100%.

Preheating helps stabilize milk exposed to high heat treatment (*41*) Biryukova et al. (*14*) tested the effect of preliminary heating on the stability of milk proteins. They concluded that for indirect UHT processing, the milk should be preheated to 75 C for 20 sec and the precipitated protein centrifuged out. Ball (*11*) suggested preheating milk in a retarder vessel at 85 C for 6 min. This would help alleviate the problem of milk-stone deposits on heating surfaces.

Homogenizing at temperatures lower than normal also reduces sediment formation. Homogenization after processing, rather than before processing, also cuts down on sediment formation (*41*). During storage, some of the sediment apparently returns to solution and thus the total amount of sediment is reduced (*21*).

Differing views have been cited, but usually indirect heating has been found to cause more sedimentation than direct heating (*21,23*). Perkin et al. (*71*), however, claimed that in none of the experiments comparing direct and indirect heating methods was any attempt made to control the severity of the heat treatment. In their experiments, when heat treatments of the same sporicidal effectiveness were given, directly-heated UHT milk gave twice as much sediment as indirectly-heated milk.

Claesson et al. (*25*) have suggested that deposit formation in UHT sterilizers could be prevented by adjusting the pH of the milk to 6.9; however, this increases the non-protein nitrogen in the UHT milk.

Gelation in UHT milk is an important problem because it signifies the final limit of storage life (*21*). The mechanism for gelation has not been well established but is probably similar to that for clotting of milk during cheese-making. The main difference is that the former occurs naturally and the latter is induced. Also, a gel is softer than a clotted curd. Sensitivity to gelation is greater with UHT processing than with sterilization in a container. It also has been reported that after being autoclaved in the bottle, milk does not gel for long periods (*79*). The relationship between gelation and sedimentation is not clear. Andrews and Cheeseman (*4*) believe that gelation is a first step towards sedimentation. They have postulated, based on molecular weight changes of casein components, that at least two processes take place during storage of UHT milk. The first process is the result of physical forces of association, such as hydrophobic bonding between the casein and lactose, which leads to the formation of a gel. The second process is the Maillard reaction where formation of covalently-bonded polymers leads to browning and sediment formation.

Researchers are still not sure whether gelation is due to enzymatic action or chemical and physical processes (*3*). Some workers have considered possibilities that a reactivated proteolytic enzyme might cause gelation (*21,79*). Extensive protein breakdown of K-casein to para-K-casein (similar to rennet action) was observed in UHT milk that had gelled due to protease (*P. fluorescens*) activity (*54*). β-Casein was also broken down rapidly while α-casein was degraded slowly. Burton (*21*) has concluded that coagulation is caused by the slow action of proteolytic enzymes (eg. from psychrotrophs such as pseudomonads), which ultimately destabilize the casein. Nakai et al. (*65*) found no evidence of proteolytic enzymes in sterile concentrated milk after a few weeks of storage although the samples had gelled. Samel et al. (*79*) also found that proteolysis was not the primary cause of gelation in UHT-milk. Some researchers (*21*) have suggested that both coagulation and development of a bitter flavor might be caused by protein changes and might be related. High heat and long holding times increase the extent of coagulation. Studies (*68,79*) have indicated that reactive sulfhydryl groups may contribute to instability of milk protein leading to gelation and/or deposit formation. Citrate delays coagulation. An inverse relationship has been observed between the degree of protein decomposition and the time of onset of gelation in UHT-milk (*79*).

Corradini (*26*) determined that the resistance of UHT-milk to gelation on storage can be increased in three ways: by reducing the flow-rate of the milk through the sterilizing equipment, by adding 0.01-0.15% disodium phosphate and by storing the product at 10 C. Varying heat treatment improves control of gelation; however, adding polyphosphates controls best (*21*).

Clotting properties

When treated with enzymes such as rennin or pepsin, UHT-processed milks do not clot as well and require twice as much time to coagulate as pasteurized milk (*21*). When Burton (*21*) suggested that UHT-milk did not clot "well", he probably meant that the curd obtained was not too firm, which would probably decrease cheese yields. Perkin et al. (*71*) found that indirect heating reduced the rate of UHT-milk clotting with rennin and pepsin more than direct heating. On the other hand, Stone et al. (*88*) reported conflicting results when they studied the effect of sterilization (113-158 C) treatment of milk on cheese starter activity and on the quality and yield of short-set cottage cheese curd. Their curd quality rated good, starter activity was 8-12% faster and yield was 4-12% higher for UHT curds than for curds from milk pasteurized at 62.7 C for 30 min. Stone et al. (*89*) confirmed that the UHT processes (115 to 157 C) that denatured up to 42% of the heat-denaturable whey proteins produced curd with flavor, body and texture similar to curd made from pasteurized milk (72 C for 30 min); also the rate of acid development was faster in the UHT curd. In a combination of two new processes, Schafer and Olson (*82*) found that UHT treatment (130 C for 2 sec versus 80 C for 2 sec) significantly increased yields and recovery of fat and solids-not-fat in Mozarella cheese made by direct acidication. Woods (*109*), in a review, found that though observations and opinions vary, many workers find that UHT treatment supports bacterial growth better than conventional pasteurization techniques. The relationship between heat treatment and the growth rate of lactic streptococci can best be described by a U-shaped curve. Oxygen expulsion, destruction of inhibitors, partial protein hydrolysis and whey denaturation were believed to be responsible for the low heat treatment stimulation (62-71 C for 30-40 min) of starter cultures, formation of toxic volatile sulfides was associated with inhibition in the middle heat treatment ranges (71 C for 45 min, 81 C for 10-45 min or 90 C for 1-45 min) and heat-induced (autoclaving or extreme heating) disappearance of sulfides resulted in stimulation of lactics at high temperatures. Woods (*109*) reported that the process of UHT-heating of skim milk to increase yields of cottage cheese has been patented.

Freezing point

It has been claimed that UHT processing raises the

freezing point of milk, leading to a false diagnosis of adulteration by water. In one study with indirectly-heated UHT milk, it was demonstrated that the freezing point remain unchanged. In other studies, it has been shown that for Uperization and vacuum pasteurization (processes where dissolved gases are removed from milk), the freezing point has been raised (*21*).

Fat separation

High temperatures of processing reduce the cream line in whole milk (*21*). The stability of fat dispersion in UHT-milk can be increased by proper homogenization. Homogenization at 211 kg/sq cm and 71-77 C with the homogenizer located down-stream from the heater will greatly reduce fat separation in UHT-milk (*41*).

Color

The appearance and color of milk primarily depend on the size gradient of the fat globules, distribution of the milk proteins and the browning reaction (*40*). Non-enzymatic browning of UHT-milk affects its flavor more significantly than its color (*41*). UHT-milk is whiter than the corresponding raw milk. Whitening is believed to be caused by denaturation and subsequent coagulation of soluble protein components of milk, which increase the amount of opaque particles in the milk (*21*). The smaller the fat globules, the whiter the color of milk because these fat globules help scatter light.

Hostetler (*40*) observed a striking difference in the color between Uperized and autoclaved milk under ultra-violet light; Uperized and pasteurized milks show yellow fluorescence while autoclaved milk shows blue fluorescence. Burton (*18,19,20*) demonstrated that UHT-milks reflect considerably more light in the 400-550 nm (violet, blue and green regions of the spectrum) range than do milks sterilized in bottles.

Texture

A chalky texture is a frequently-mentioned shortcoming of UHT processed milks, particularly those processed by direct heating methods. This is apparently an early stage of sediment formation. Homogenization after heat treatment alleviates this (*21*).

FLAVOR

The flavor of UHT-milk is only slightly different from pasteurized milk (*40*). At higher processing temperatures, the aromatic substances of the milk which cause odors such as feedy or barny are removed more efficiently. Thus, consumers find the UHT-milk to be flat or "purer." On the other hand, some consumers also criticize the UHT-milk for off-flavors, such as cooked and stale.

In the direct-heating UHT processes where steam is injected into milk, or vice versa, the condensed steam has to be removed. This is accomplished when the milk is vacuum-cooled by flashing it into an expansion vessel under vacuum. Besides moisture, a major portion of the aromatic components, the sulfhydryl groups (SH) and oxygen also are removed. This deaeration helps retard the changes induced by oxygen. Cooling by flashing also is used after some indirect UHT processes; then the amount of moisture removed is returned to the milk (*40*).

Hansen et al. (*38*) processed milk at several temperatures between 107-143 C and stored the milks at 1.7 and 7.2 C in polyethylene bags or clear glass containers. The milks, evaluated by a 25-member panel at 2, 9, 16, 23 and 30 days, were judged from acceptable to good. The cooked flavor disappeared in 9 days and gave way to a slightly sweet flavor. In general, the flavor was most acceptable at 9-16 days.

Ashton (*9*) classified the general flavor changes in UHT-milk packaged in waxed paper and polythene laminate cartons and stored at between 4 and 22 C into 5 periods: (*a*) Period 1, immediately after processing. Unpleasant taste and smell, hydrogen sulfide, carbon disulfide and boiled cabbage flavors. (*b*) Period 2, at 2-3 days. Weaker hydrogen sulfide, cabbage and less unpleasant flavor, slight residual "cooked". (*c*) Period 3, at 5-12 days. Best flavor is at this stage, traces of initial unpleasant flavor, creamy taste similar to pasteurized milk. (*d*) Period 4, at 12-18 days. Appearance of flat, chalky or slight residual cooked flavor. (*e*) Period 5, at 19 days. Slight development of incipient oxidative rancidity or "cardboardy", becoming progressively more obnoxious with age.

According to Ashton (*9*), all the above stages progress faster if higher storage temperatures are used. The time necessary to pass through each stage does not depend on the thickness of the carton's internal polyethylene layer. A black lining also does not affect the rate at which off-flavors develop; however, an aluminum foil lining helps increase the duration of each phase. The off-flavors that Ashton associates with Period 5 (incipient oxidative rancidity, "cardboardy") are probably what some other researchers (*29,96,105,106,107*) call stale.

A U.S. committee on Flavor Nomenclature (*85*) has hypothesized that there are four kinds of heat-induced flavors: cooked or sulfurous, heated or rich caramelized and scorched. Milk when heated to 135 to 150 C for several seconds exhibits a strong sulfurous or cooked flavor; after several days of storage this flavor disappears to leave a rich or heated note. Volatile sulfides are believed to contribute to the cooked flavor and it has been suggested that nonenzymatic browning causes the caramelized flavor but the compounds responsible for the rich or heated note have not been clearly elucidated. It is possible that what many researchers refer to as "stale" (*29,96,105,106,107*) is a combination of "rich or heated" and "caramelized". A method of preparing reference standards for these flavors has been presented (*85*).

Cooked flavor and sulfhydryls

The cooked off-flavor is one of two primary flavor criticisms of UHT milk. Cooked flavor is first noticeable when raw milk is heated momentarily to about 75 C or when it is exposed to lower temperatures for a prolonged time (*42*). Heat liberates volatile sulfides and sulfhydryls and lowers the redox potential. The sulfides and

mercaptans result from the heat denaturation of only the serum proteins (*42*). β-Lactoglobulin, the major component of the albumin fraction of the serum proteins, can account for almost all the volatile sulfur-bearing compounds. The susceptibility of generic variants of β-lactoglobulin to heat denaturation are in the order C > B > A (*81*). The volatile sulfur-bearing compounds probably originate from methionine, cysteine and cystine which are the only common amino acids containing sulfur. The probable mechanism of the cooked flavor involves conversion of those amino acids to hydrogen sulfide and methyl sulfide (*69*). Cabbagey defects in UHT-milks have been correlated with hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide and carbon disulfide (*43*).

Blankenagel and Humbert (*16*) showed that in the 82-140 C range, the primary effect of the increase in temperature on skim milk was denaturation of serum proteins; β-lactoglobulin is completely denatured at 130 C. They also reported that denaturation yields sulfhydryls which cause the cooked flavor. After a week of storage at room temperature, the volatile sulfur compounds were completely gone; but at 4.5 C, the rate of disappearance was much slower. This observation was confirmed by Lyster (*56*) for UHT-pasteurized milk stored in screw cap bottles. Burton (*20*), Hostetler (*40*) and Ashton (*9*) pointed out that UHT-milk had a hydrogen sulfide odor and a cooked flavor immediately after processing but they disappeared within 24 h after processing.

Patrick and Swaisgood (*68*) found that the reactive sulfhydryl groups in UHT-skim milk were oxidized more rapidly at room temperature than at refrigeration temperature. Concentrations of reactive sulfhydryls in UHT-milk were correlated with the undesirable "cooked" flavor, and it was speculated that these could contribute to instability of milk protein through disulfide interchange reactions.

Hostetler (*40*) cited evidence to show that the decrease in concentration of sulfhydryl compounds is accelerated by exposure to light and higher storage temperatures. Milkfat retards the loss of sulfhydryl compounds, and whole milk loses sulfhydryl compounds more slowly than does skim milk. Summer milk resists loss of sulfhydryl compounds better than winter milk.

It has been suggested that the differences in SH stability at different heating temperatures, holding times, and storage times are due to the enzyme sulfhydryloxidase which oxidizes the SH groups linked to protein compounds (*56*). Sulfhydryloxidase has been isolated from raw milk, purified and characterized by researchers at North Carolina State University (*44,91*); furthermore, they have developed a method of immobilizing the enzyme on a laboratory scale.

Jordan (*48*) confirmed the cooked flavor of milk disappeared within a few days because of oxidation. In the direct UHT process, oxidation occurs more slowly because oxygen is removed by vacuum cooling. When oxygen is passed through the product, oxidation of the reducing substances is accelerated. This process has been patented by AB Tetra Pak in Sweden (*40*).

Zadow et al. (*112*) tested the influence of anti-oxidants (10 mg of butylated hydroxy-anisole + 10 mg of tocopherol + 50 mg of Tween 80 per kg of milk), storage time (1-week interval for 3 months), storage temperature (2 or 20 C), and re-examination 2 days after opening on the flavor acceptance of UHT-milk (150 C for 6 sec or more) stored aseptically in cans. Statistically, the presence of antioxidants was the most important factor; three of four panelists preferred milk with antioxidants, the fourth objected to the metallic taste imparted by the antioxidants.

Ferretti et al. (*29*) recently recommended a sulfhydryl blocking agent to control cooked flavor in sterile milk concentrates. The blocking agent used was 2-acetamidoethyl-2-acetamidoethane-thiolsulfonate at the rate of 3.8 mg/100 ml of reconstituted milk. Results indicated that if this blocking agent is added after the sterilization process, the milk flavor is more desirable - especially during the early days of storage at 4 and 21 C. As the storage time increased, however, the differences between the control and the treated samples narrowed, and the milks were criticized as stale, scorched or typical evaporated milk. Samuelsson (*80*) has patented use of 30 ppm of potassium iodate, and Food Innovation AB (*32*) has patented use of 5-70 ppm of sodium iodate, sodium bromate, potassium iodate or potassium bromate to inhibit formation of SH groups and thus improve the flavor of UHT milk. Badings (*10*) found that 30-70 mg of L-cystine added to every kg of milk before UHT sterilization reduced cooked flavor intensities and hydrogen sulfide concentrations.

Stale

Besides cooked, the other common flavor criticism of UHT milk is "stale." Two basic approaches have been used to study the stale flavor. The first involves elucidating the mechanism, and the second involves identifying the compounds associated with the flavor. The stale flavor becomes noticeable after the cooked flavor starts to diminish (*29,96*).

Schmidt (*83*) found that off-flavors developed in UHT-milk within 3-6 weeks at 20 C and 1 week at 38 C. UHT-milk of reduced fat content (1.7%) showed less sensory change during storage. Storage of sterilized milk in daylight caused sensory deterioration but did not affect free fatty acids and hydroxymethylfurfural content. On the other hand, Langsrud and Hadland (*53*) found that when UHT-sterilized goat's milk was stored at 4, 20-25, 30, 37 and 50 C, maximum increase in non-protein nitrogen was at 50 C accompanied by a bitter flavor (within 7 days), increased 5-hydroxymethylfurfural content, reduced lysine and distinct browning. At all temperatures except 4 C, there was progressive browning related to temperature, along with destabilization of fat and some sedimentation — but no gelation.

According to Thomas et al. (*96*), some investigators

associated the stale flavor with products of the Maillard reaction. 1-Amino-1-deoxy-2-ketohexose was identified as the stale principle in sterile milk concentrate (*96*). This is a product of the Maillard reaction and thus, browning and staling were related.

In a series of experiments (*105,106,107*), Whitney's research group proved that a stale-flavor component, which develops in dried whole milk upon storage, was present in the butter oil fraction. They also established ideal conditions for a steam distillation procedure by which the stale component could be distilled and trapped in fresh butter oil or distilled water.

In a recent series of papers, Moller et al. (*61,62,63*) studied chemical changes in UHT-milks during storage. Caseins from stored UHT-milks resisted proteolysis better than casein from unheated milk (*61*). This resistance was attributed to the Maillard reaction between milk proteins and lactose during storage of UHT-milk. The first stable intermediate (*62*) of this reaction was identified as lactuloselysine (ε-N-deoxylactulosyl-L-lysine) and its hydrolytic breakdown product fructoselysine (ε-N-deoxylactulosyl-L-lysine). Indirect evidence for their formation was obtained when Finot et al. (*30*) identified furosine and pyridosine in roller-dried milk powder. Among UHT-milks stored at different temperatures for different times, browning was most apparent at the highest temperature for the longest time (37 C for 3 years) and in this, only part of the lysine residues was accounted for as lactuloselysine and fructoselysine. Thus lysine had become involved further in sugar degradation products.

The volatile chemical compounds identified in a number of different kinds of stored milk products have been reviewed by Arnold et al. (*8*). The compounds identified are n-aldehydes (C_1-C_3, C_5-C_{10}, C_{12}, C_{14}), n-methyl ketones (C_3-C_5, C_7, C_9, C_{11}, C_{13}, C_{15}), n-fatty acids (C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}), 2-pentanone, 2-hexanone, 2-heptanone, δ-decalactone, δ-dodecalactone, furfuraldehyde, benzaldehyde, 2-methylheptanal, o-aminoacetophenone acetone, pentyl acetate and dimethyl sulfide. In experiments with stale, sterile concentrated milk, Arnold et al., identified 2-heptanone, 2-nonanone, 2-tridecanone, benzaldhyde, acetophenone, naphthalene, a dichlorobenzene, δ-decalactone, benzothiazole and o-aminoacetophenone. Of these compounds, only the dichlorobenzene and 2-heptanone were identified in the control milk. Control milk was stored at 1 C and the experimental milk at 22 C. More recently Jeon et al. (*47*) observed increases of acetone, methylketones (C_3, C_5, C_7, C_8, C_9, C_{11}, C_{13}), n-aldehydes (C_3, C_5, C_6, C_7, C_8, C_9) and 1-butanol in UHT (145 C for 3 sec)-milks stored at 3, 22 and 35 C for 5 months. Although the methyl ketones were most abundant, aldehydes appeared to be the significant contributors to the off-flavor of stored UHT milk. Oxygen in milk affected concentration of only the aldehydes, whereas storage temperature affected concentrations of aldehydes and the methyl ketones. Some observations of Jeon et al. were corroborated by Mehta and Bassette (*58*). Increase in stale off-flavor intensity for UHT-milk stored at 22 C occurred concurrently with increases in propanal, pentanal and hexanal and decreases in cooked flavor and methyl sulfide. No lipid oxidation was observed and although some relationship was observed between browning and UHT heat treatment, changes during storage were significant only during the first period (12 days) of storage. Mehta and Bassette (*58*) suggested that development of staling as cooked flavor disappeared suggested that the mechanism for staling depends on the oxidation-reduction potential. Refrigeration at 4 C helped retard but not eliminate the rate of increase of the aldehydes and stale off-flavor (*59*). Hostetler (*40*) confirmed that UHT-milk resisted formation of oxidized flavors better than did pasteurized milk because of reducing substances. These reducing substances are probably sulfhydryl in nature. Keeney and Patton (*49*) also associated some lactones with off-flavor in stored milk products. The flavor they described as non-oxidative was probably what other researchers referred to as "stale." However, Lyster (*56*) associated δ-dodecalactone and δ-dodelactone and Keeney and Patton (*49*) associated δ-decalactone with a coconut-like off-flavor in stored milk products.

Morgan et al. (*64*) patented a process for adding .002-0.4% of a citrus bioflavonoid, preferably hesperidin, to a milk product before sterilization. They claim that these bioflavonoids prevent stale flavors in sterilized canned milk products during storage.

Oxygen

Zadow (*110*) observed that after storage for short periods, UHT-milk with a high oxygen content was preferred. After storage for prolonged periods, the flavor of milk with low oxygen was found to be better. The deterioration in flavor resulted in oxidized or slightly rancid off-flavors. The two main factors that affect development of off-flavors in UHT-milk are level of oxygen and temperature of storage (*111*).

Tarassuk (*93*) studied the effect of oxygen content in the head-space of cans sterilized by autoclaving at 85-120 C. A correlation was found among carbon dioxide produced, oxygen depleted, the intensity of cooked flavor, and the degree of browning discoloration (measured by sensory evaluation). The data suggest that the flavor and color of sterilized milk may be improved by lowering the available oxygen in a can before sterilization. This also suggests that if the oxygen in the milk during UHT processing is limited, milk with a better flavor can be produced.

Thomas et al. (*96*) experimented with the effect of oxygen content on the flavor of indirectly-heated UHT-milk during a 150-day storage period at room temperature. The UHT-milk was prepared with initial oxygen contents of 8.9, 3.6 and 1.0 ppm which represent almost the entire range of concentration expected under normal processing conditions. Flavor acceptability was maximum at 6 days, after which it slowly declined. The

increase in acceptability from 0 to 6 days was associated with the decrease in the off-flavor described as cabbagey; after 6 days, the milks started acquiring a stale characteristic. Up to 8-13 days, the higher the initial oxygen content, the more acceptable a milk was; however, after this, the acceptability did not depend on initial oxygen content. Losses of sulfhydryls, ascorbic acid and folic acid were less with lower initial oxygen and vice versa, but the benefits of high oxygen content on flavor were slight and were outweighed by the adverse nutritional effects.

Zadow and Birtwistle (*111*) derived a relationship between the level of dissolved oxygen in a product and the head-space volume of a sealed container. The total mass of oxygen in a container can be calculated if the filling temperature, head-space volume, pressure of oxygen in the milk and the mass of the milk are known.

Packaging

The ideal packaging material should be odor-free, non-reacting, sterile and nearly impermeable to air and light (*31*). Hostetler (*40*) has reviewed the effect of packaging on the flavor of milk. Frequently plastic foils used in the dairy industry have an odor. For instance, polyester foils (polyethylene terephthalate) may have terephthalic acid which has a paper-like odor; polyethylene foil, an odor of oxidized oil and wax; polypropylene foil, a burnt and phenolic odor.

Hansen et al. (*38*) cited evidence to show that when UHT-milk was packaged in a polythene bag and cardboard box, the sizing glues and components in the cardboard were absorbed through the polyethylene (PE) films. The resultant cardboard flavor was eliminated when sterile glass containers were used.

Bojkow (*17*) tested the effect of PE-laminates on the flavor of milk and water. He concluded that oxidation of the PE-film was the most common cause of PE-related off-flavors. Some stale, bitter and astringent flavors in milk were shown to emanate from the PE-film. These were more intense in the water than in the milk. The intensity of off-flavors transferred from the film decreased when the package materials were stored for a long time prior to use.

Flückiger (*31*) compared 1-liter PE-coated cartons with (AC) and without (PC) aluminum foil. The AC did not lose any weight during a six-week storage period. During the same period, the PC lost about 0.2% of the original weight during storage at 20 C and about 1% at 38 C. The oxygen in the milk in AC remained almost unchanged (1 ppm); however, in PC, the milk was saturated with oxygen (8 to 9 ppm) after a few days. Most of the oxidative changes in the PC took place in the first 2 or 3 days after processing. In other words, the reducing substances in the milk that offer protection against oxidation decreased rapidly in PC cartons. This milk was organoleptically acceptable up to 3 weeks when stored at 15 C. The AC milk was organoleptically acceptable up to 2 months even at a storage temperature of 38 C. Mehta and Bassette (*58*) confirmed that UHT-milk in AC cartons retained a desirable flavor longer than that in PC cartons, at least partly because PC cartons were more permeable to gases. Wrapping either carton with Saran and aluminum foil was detrimental to flavor, probably because the wrapping not only excluded outside air from the milk but also served to exclude volatile compounds coming from either growth of microorganisms on the surface of the cartons or from the wrapping material itself.

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Aflatoxin M in Perspective[1]

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ABSTRACT

The steps are presented from the discovery of aflatoxin M_1 in the milk of a cow fed "toxic" peanut meal to the determination of its relation to aflatoxin B_1, and the relation of aflatoxin M_1 to other animal metabolites of aflatoxin B_1. Estimates from controlled studies are given of the level and amount of aflatoxin M_1 to be expected in the milk of cows exposed to aflatoxin B_1 in their feed, followed by numerous survey results that corroborate the conclusion from the experimental estimates: levels of aflatoxin B_1 in uncontrolled dairy ration ingredients are sufficiently high to result in measurable aflatoxin M_1 (> 0.1 ng/ml) in commercial milk supplies. Because the Food and Drug Administration has been unable to prevent occurrence of aflatoxin M_1 in milk in the United States by attempting to control the feed, an administrative guideline has been established at 0.5 ppb for aflatoxin M_1 in fluid milk products; consideration is being given to related guidelines for products manufactured from milk.

Scientists are naturally inquisitive, so it was not surprising that soon after a lot of Brazilian peanut meal was found to be associated with the 1960 Turkey X disease, Allcroft and Carnaghan (*1,2*) decided to determine the effect of the "toxic meal" on dairy cattle and poultry. These two investigators in the Central Veterinary Laboratory at Weybridge, England, where much of the original work on Turkey X disease had been conducted, particularly wanted to know whether any of the toxin was transmitted to those animal products used as food. By the time their investigations were concluded in 1962 the toxic compounds had been isolated, named "aflatoxins" and partially characterized, but the analytical method of choice was still a biological test -- observation of the degree of bile duct hyperplasia induced in day-old ducklings dosed with the toxic product. (Subsequent chemical analysis in 1966 of the toxic meal used (*3*) showed aflatoxin B_1 and B_2 levels of 10 and 0.2 μg/g, respectively.) Dairy cattle were fed a concentrate ration containing 20% toxic meal; layers were given a ration containing 15% toxic meal. Histological evidence of toxicity was found in the livers of ducklings dosed with milk from the cows given the concentrate with toxic meal; there was no evidence of toxicity in the milk from control cows. The liver from a cow that had consumed toxic meal, and eggs from layers that had consumed toxic meal were similarly tested, with no evidence of toxicity.

In 1964 the focus of the action shifted to the Unilever Research Laboratory in Vlaardingen, The Netherlands (*8*), another of the original Turkey X investigating groups. Using dried toxic milk supplied by the Weybridge laboratory, de Iongh et al. (*8*) were able to show that the toxicity resided in a fraction of the milk extract which was chromatographically different from the aflatoxins present in the peanut meal. A compound chromatographically similar to the "milk toxin" was also found in an extract of a culture of *Aspergillus flavus,* the mold that had been associated with the toxicity. To determine whether the milk toxin was the result of direct transmission of the mold-derived product or the result of metabolic conversion of the major aflatoxin in the peanut meal, pure aflatoxin B_1 was given to lactating rats. The appearance of the typical fluorescent spot in chromatograms of extracts from the milk of dosed rats showed that the lactating rat was able to convert aflatoxin B_1 into the "milk toxin." The coincidence of the biological and chemical tests for the milk toxin was established by the same research group (*34*).

With a sensitive chemical detection method now available, further experiments with rats dosed with pure aflatoxin B_1 (*5*) showed that conversion to the milk toxin was taking place in the liver and that the appearance of the milk toxin in the liver and systemic blood coincided within hours with the disappearance of the aflatoxin B_1, after which, over a period of days, the milk toxin also disappeared. The chromatographic reference standard for this work was an extract of the "toxic milk" supplied by the Central Veterinary Laboratory, Weybridge.

Curiosity further impelled the group at Weybridge to examine the metabolism of aflatoxin in sheep (*5*), at that time considered an aflatoxin-resistant species. With the assistance of colleagues at the Tropical Products Institute, they found the milk toxin present in the liver, kidney and urine of sheep given a crude extract of mixed aflatoxins either intraperitoneally or by stomach tube. At this time they recommended use of the trivial name "aflatoxin M" and suggested that urine of dosed sheep might provide a convenient source of product for preparatory isolation.

This last suggestion was adopted by the Council for Scientific and Industrial Research laboratories in Pretoria, South Africa, where the observation was confirmed and sufficient aflatoxin M_1 and M_2 (corresponding to B_1 and B_2) was isolated in pure form for determination of their structures (*11*). The same compounds were also isolated from moldy peanuts. To clinch the identity of the urinary compound with the milk

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toxin, a research group at the Western Regional Research Laboratory of the U.S. Department of Agriculture isolated crystalline aflatoxin M_1 from the urine of sheep and from the milk of a cow dosed with aflatoxin from an *A. flavus* culture on rice (*21*). The identity of the aflatoxin M_1 from the urine and from the milk was established on the basis of melting points, ultraviolet spectra, formation of acetyl derivatives, crystallography, X-ray diffraction powder patterns, infrared spectra and paramagnetic resonance spectra. The structure for aflatoxin M_1 derived by the Pretoria group from their data was confirmed as aflatoxin B_1 hydroxylated at the tertiary carbon of the difuran ring system (Fig. 1).

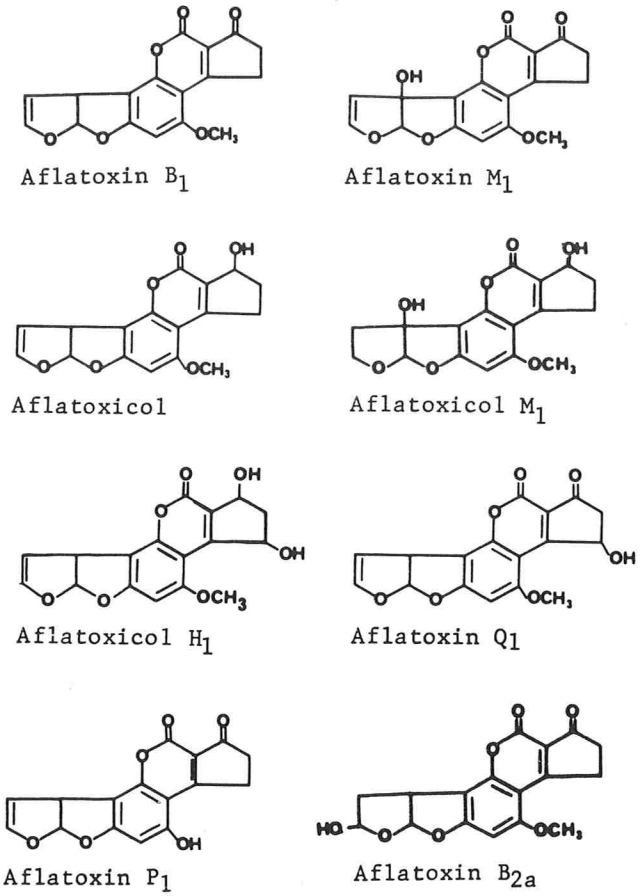

Figure 1. *Chemical structures of aflatoxin B_1 and identified products of its metabolism by animals.*

AFLATOXIN METABOLISM

It is of value at this point to place aflatoxin M in the total concept of aflatoxin metabolism as it is presently understood (*7,12*). Aflatoxin B_1 administered to animals disappears at widely different rates, depending on the animal species. Only a very small portion of the administered aflatoxin is found unaltered in the excretions or secretions of the animals tested. Most of the aflatoxin is altered by the cytoplasmic and microsomal enzymes in the liver cells to form oxidation or reduction products. The reduction product, aflatoxicol, and the numerous oxidation products in addition to aflatoxin M_1 have been characterized (Fig. 1). All of these alterations result in the addition of one or more hydroxyl groups to the molecule. Conjugates of some of these hydroxyl derivatives with sulfate, glucuronic acid, and protein have been identified; conjugates of this type have enhanced water solubility, and reduced solubility in solvents such as chloroform. Since studies with ring-labeled aflatoxins administered to animals show a large portion of the radioactivity in the water-soluble fractions of the excreta (*7,19,30*), the assumption has been made that much of the administered aflatoxin is eliminated as a conjugate, analogous to the elimination mechanism for many other toxic substances. There has been no attempt, so far, to measure the relative amounts of hydroxylated aflatoxins produced by a cow, or to determine whether any of them, other than aflatoxin M, or their conjugates can be secreted in the milk.

From the original observation that milk from cows exposed to aflatoxin B_1 caused bile duct hyperplasia in the duckling, it is obvious that the metabolic alteration had not eliminated the aflatoxin toxicity or the typical lesion in the duckling. Aflatoxin M_1 acute toxicity in the day-old duckling was found to be approximately the same as that of aflatoxin B_1 (*28*); a similar coincidence of acute toxicities was found in the rat (*37*). However, the carcinogenic potency of aflatoxin M_1 was found to be much lower than that of aflatoxin B_1 in both the rat (*37*) and rainbow trout (*6,31*). Some quantitative measure of the relative mutagenic potency of aflatoxin B_1 and the products of its metabolic conversion was obtained by the Ames procedure, using a *Salmonella typhimurium* mutant assay, including activation of the compounds with an enzyme preparation from rat livers (*38*) (Table 1). According to this study, aflatoxin M_1 has about 3% of the mutagenic potency of B_1, approximately the same as aflatoxin G_1. Except for aflatoxicol, with 23% of the potency of aflatoxin B_1, all the other metabolites were weaker mutagens than aflatoxin M_1.

AFLATOXIN IN MILK

Results from seven controlled feeding studies show that the proportion of aflatoxin B_1 that is ingested by a cow and appears as aflatoxin M_1 in the milk, calculated as the pooled average, is approximately 1% (*14*), with a

TABLE 1. *Relative mutagenic potency of aflatoxins by the Ames mutant Salmonella typhimurium test with activation of test compounds by rat liver cell enzymes (from ref. 38).*

Aflatoxin	Relative mutagenicity
Aflatoxin B_1	100
Aflatoxicol	23
Aflatoxin G_1	3
Aflatoxin M_1	3
Aflatoxicol H_1	2
Aflatoxin Q_1	1
Aflatoxin B_2	0.2
Aflatoxin P_1	0.1
Aflatoxin G_2	0.1
Aflatoxin B_{2a}	0
Aflatoxin G_{2a}	0

range of 0.4 to 3%. An observation better suited to control and regulatory purposes is that an aflatoxin B_1 level of 300 ng/g in the feed could result in a 1 ng/ml level of aflatoxin M_1 in the milk. This ratio is obtained from the average of eight different herds that had been used in the reported studies. The herd sizes ranged from 1 to 10 cows and the aflatoxin B_1/M_1 level ratios for the herds ranged from 46 to 646. Aflatoxin M_1 appeared in the milk within 24 h of exposure of the cows to aflatoxin B_1 and dropped below detectable levels 4-5 days after withdrawal of the contaminated feed (*4,14,20,22,26,34*). The time until disappearance of the aflatoxin M_1 from the milk bore no relation to the level of aflatoxin in the feed or the level in the milk before withdrawal of the contaminated feed.

ANALYTICAL METHODS

To carry out the aforementioned studies it is obvious that analytical methods were required, and considering the variety and differences in sophistication of the methods used, it is obvious that the spread in results observed could have been due as much to analytical methods as to biological variability and study detail. The first methods were relatively crude; the next generation of methods became more complicated in the search to lower the limit of detection to almost 0.1 ng/ml. These methods are exemplified by the first two adopted by the Association of Official Analytical Chemists (AOAC) for analysis of aflatoxin M_1 in milk, one for fluid milk (*23*) and another for milk powder (*24*). A more simple, general method for aflatoxin M_1 in dairy products (*25*) has since superseded the first two methods. This last method has been used for most of the survey work in the United States. An even simpler general method has now (June 1979) been validated by an international collaborative study sponsored by the AOAC and the International Union for Pure and Applied Chemistry. With the current generation of analytical methods, an aflatoxin M_1 level in fluid milk of 0.1 ng/ml is a practical limit of determination for routine work, with a limit of 0.02 ng/ml achievable under research circumstances by an experienced analyst.

AFLATOXIN IN MILK PRODUCTS

Considering the limit of determination of the analytical methods, the conversion ratio for aflatoxin B_1 in the feed to aflatoxin M_1 in the milk, and the potential contamination of some dairy ration ingredients with aflatoxins, it should be no surprise that when surveys were carried out, aflatoxin M was found in commercial dairy products in a number of countries (*13,18,27,29,36*) (Table 2). In the 1973 survey in the United States (*29*), the geographic distribution of contamination was clearly related to known sources of aflatoxin-contaminated feed ingredients. A number of the studies of German milk production (*17,18,27,29*) showed a positive relation between the incidence of aflatoxin M in milk and the period during which supplemental feeds are used, including imported oilseed meals.

The unusually heavy aflatoxin contamination of the 1977 corn crop in the southeastern United States produced further evidence of a relation between the occurrence of aflatoxin contamination in a major dairy feed ingredient and the appearance of aflatoxin M in milk. The severity of the feed contamination is reflected in the incidence and levels of aflatoxin M found in the milk (Table 3). Aflatoxin M was detected in 63% of all the Southeast samples, in 80% of the samples from the state of Georgia, and in one or more of the samples from 84% of the bottling plants represented by the samples taken. The survey also produced the highest reported level, up to that time, of aflatoxin M_1 in a commercial milk sample in the United States, 4 ng/ml.

A more recent episode of aflatoxin contamination of milk occured in Arizona in July 1978. It started with a huge pile of Arizona cottonseed with total aflatoxin levels

TABLE 2. *Reports of aflatoxin M_1 in commercial dairy products.*

Country	Year	Types of samples examined	No. of samples examined	Percent positive	Range of levels found (ng/ml)[a]	Ref.
South Africa	1968	Market milk	21	24	0.02-0.2	*29*
United States	1973	Cottage cheese, nonfat dry milk, evaporated milk	320	8	0.05-0.5	*29*
Belgium and The Netherlands	1975	Raw and market milk	68	62	0.01-0.5	*36*
West Germany	1971	Dry milk products	166	5	0.07-0.2	*29*
	1972	Raw and market milk	61	46	0.04-0.25	*13*
	1972-3	Nonfat dry milk	120	62	0.02-0.4	*29*
	1972-4	Fluid milk	260	45	0.05-0.3	*27*
		Nonfat dry milk	41	73	0.02-0.2	*27*
		Yogurt	54	82	0.05-0.5	*27*
	1976	Raw milk	419	19	0.05-0.54	*18*
United States	1974-5	Cheese imported from Europe	156	8	0.1 -1.0[b]	*29*
West Germany	1972-4	Various cheeses	356	48	0.1 -1.3[b]	*27*
	1976	Various cheeses	197	69	0.02-0.23[b]	*17*

[a]All levels reported on fluid milk basis.
[b]ng/g.

TABLE 3. *Distribution of aflatoxin M_1-contaminated fluid milk samples taken in the period 31 October — 3 November 1977, by contamination level and state where bottled.*

Aflatoxin M_1 range (ng/ml)	Alabama		Georgia		South Carolina		North Carolina	
	No.	%	No.	%	No.	%	No.	%
ND[a]	44	57	15	20	30	40	22	29
T[b]-0.2	22	29	38	51	28	37	38	51
0.3-0.4	7	9	16	21	11	15	12	16
0.5-0.7	3	5	2	8	6	8	3	4
>0.7	1		4		0		0	
Total number of samples	77		75		75		75	

[a]ND = none detectable.
[b]T = trace.

in the μg/g range and an unhappy set of circumstances that spread the contaminated seed to dairy farms in Arizona and six adjacent states before State and Federal regulatory authorities could control the situation.

CONTROL

The initial Food and Drug Administration (FDA) approach to control the occurrence of aflatoxin M in milk had been to control the aflatoxin B_1 contamination of feed. The FDA action guideline of 20 ng/g for total aflatoxins ($B_1 + B_2 + G_1 + G_2$) in all feeds or feed ingredients, if observed, should preclude detectable aflatoxin M in milk. Even before the occurrence of the severe aflatoxin-in-corn problem in the Southeast in 1977, it was obvious that cooperation by the individual states would be needed to control aflatoxin in feed, because a large proportion of feed and feed ingredients never enters interstate commerce. But even with state cooperation, there is no control of the feed that never leaves the farm on which it is grown, as revealed by the 1977 Southeast problem. When a survey of market milk in the Southeast in the fall of 1977 (Table 3) showed that voluntary and state controls of feed were not effective in preventing contamination of the milk, the Commissioner of Food and Drugs determined that the FDA regulatory program should be expanded to include milk. He therefore established an action level of 0.5 ng/ml (0.5 ppb) for aflatoxin M_1 in fluid milk products (*9,10*), taking into account both the effect of the action level on the supply of milk and data on the health effects of aflatoxin M_1. The major health considerations were: milk can constitute the major nutrient of the rapidly growing young; the rapidly growing young are, theoretically, at the most vulnerable stage for induction of carcinogenesis; in the absence of firm knowledge on the degree of risk, exposure to "unavoidable" carcinogens should be kept to the lowest level practically achievable. Since the Commissioner set an "action level" and not a "tolerance," he considered that the controlling circumstances could change rapidly. The determination of the lowest level practically achievable could change with the experience now being accumulated.

The FDA regulatory activity is by law confined to milk in interstate commerce, a few steps removed from the key control point on the dairy farm. The Federal action level does, however, provide guidance to the individual states, a number of which have adopted that action level number and established control sampling at tanker stations, a collection spot close enough to the farm to provide the desired control. Dairy cooperatives are also developing control procedures.

REMOVAL OF AFLATOXIN FROM MILK

There is currently no known procedure for destroying aflatoxin M in milk without destroying the milk. For all practical purposes, aflatoxin M is stable in raw milk and processed milk products, and is unaffected by pasteurization or processing of milk into cheese or yogurt (*15,16,32,35*). Milk products made by evaporation of water will obviously have aflatoxin M levels related to those in the raw milk from which they were made and in proportion to the concentration achieved. Products made by separation of the milk components (cream, butter, cheese, whey) show a distribution effect caused by the insolubility of the aflatoxin in the milkfat and adsorption of the aflatoxin to the curd. Aflatoxin M_1 has been found to be approximately equally distributed between the curd and whey fractions obtained in making cheese (*16,33,35*). Because of the relative amounts of curd and whey obtained and the loss of moisture during curing, the cheese can be expected to have 3.5-5 times the aflatoxin M level in the milk from which it was made. Butter will contain aflatoxin M_1 in proportion to the occluded whey component, which in turn can be expected to have an aflatoxin M level 0.5-0.7 times the level in the milk.

Obviously, these manufactured dairy products are more accessible to Federal control than is the raw milk from which they were made. The possibility of applying control measures at this level is currently under FDA review.

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yzing numerous dairy foods for fat, protein, lactose, and total solids. Procedures for sample preparation and analysis need refinement. (Mr. R. Case, Laboratory Controls Manager, Quality Assurance, Kraft Inc., 500 Peshtigo Court, Chicago, IL 60690; phone: 312-222-2826).

9. *Dilution Water System.* A dilution water system that is either non-toxic or only minimally toxic to the microbial flora in dairy foods needs refinement. The variations in water supplies suggest continual evaluations and improvements to assure microbiologically suitable diluents (Dr. R. T. Marshall, Department of Food Science and Nutrition, 203 Eckles Hall, University of Missouri, Columbia, MO 65201; phone: 314-882-7355).

10. *Other Studies.* Although the Technical Committee has identified the foregoing nine items as worthy of investigation, proposals dealing with other studies relating to standard methods for examining dairy products also will be considered. Such proposals should be sent to Dr. Bodily.

G. H. Richardson
Chairman
Technical Committee on Standard Methods for the Examination of Dairy Products

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Maximizing Efficiencies in the Food System: A Review of Alternatives for Waste Abatement

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ABSTRACT

The biological and technological inefficiencies in the total food system are examined. Energy from sunlight is converted into human food with rather poor efficiency, as only a small portion of the energy available is recovered as food. Maximum efficiency in energy flow is obtained if humans consume plants for food rather than feeding animals for production of human food. The waste generated at each stage in food production and processing is examined and waste abatement alternatives are presented. These alternatives include: changing consumer demand to accept food that generates less waste during production, changing production and processing cycles to be more efficient or accommodate waste products in the environment through conversion to useful products or classical treatment as sewage. Recent advances in reducing the environmental impact of food processing wastes using these technological alternatives are examined. Emphasis is placed on alternatives which maximize overall efficiencies in the food system.

Food producers and processors have tried to maximize the value of food output in relation to the costs incurred. In the past this was done with little regard to the environmental and social costs or consequences (*64*). This philosophy and man's inventiveness have resulted in appearance of thousands of new food products which consumers have eagerly purchased. Since environmental and social restraints could be largely ignored, cost inputs from these two sources were negligible, and production at relatively low costs could be maintained. This has led to several practices with both beneficial and detrimental results. For example, increased production efficiency could be obtained by use of fertilizers and pesticides (*15*) with little apparent regard for environmental discharges. Food processors, usually producing wastes with less recalcitrant properties, also contributed to the total 'waste load' of food from production to consumption.

While consumers were anxious to receive the maximum number of goods and services at minimum costs, certain members of society became concerned at social and environmental costs not previously considered. The popular awakening of the public, both in the U.S. and internationally, to the price of cheaper (*64*) goods and services without regard to environmental pollution has led to laws passed by the U.S. Congress which severely curtail past practices and will ultimately increase the cost of goods and services to the consumer. New attitudes must be developed by every sector of agriculture to learn to live with these new constraints (*79*). Society must also become obliged to live in harmony with its essential food producing and processing systems. It should be the national goal to accomplish this harmony at modest cost to consumers and producers as well.

The role of scientific research in this area is to discover alternative processing techniques which result in the maximum benefit to all with minimum serious tradeoffs. The waste production and utilization problem has recently been reviewed by several authors. These reports emphasize the waste load produced (*39,88*), the alternatives available (*4,5,20,26,28,30,43,59,70*) or the relative costs of waste treatment (*42,81*). This review will concentrate on maximizing the efficiencies of the food system as a whole by minimizing waste at each step. Emphasis will be placed on those processes which reduce the impact of food wastes on the environment through product or by-product recovery and recycling.

EFFICIENCY OF FOOD PRODUCTION

Production, processing, and consumption of food result in some degree of waste at each stage (Fig. 1). We are faced with certain constraints, at least at present, on the maximum efficiency of food production. If we were to maximize biological production and food processing efficiencies and consume all we produce, we would have minimum waste. Deviations from maximum efficiency during production, processing and consumption result in waste production and require more energy inputs to alleviate environmental damage, thus further reducing efficiencies and increasing costs. To understand the relative magnitude of production and utilization efficiencies, it may be useful to examine the production of one food, corn, from planting to consumption. The Committee on Agricultural Production Efficiency from the National Academy of Sciences had developed this illustrative example (*2*).

Sunlight is a free and plentiful source of energy, but only about 45% of the energy spectrum is of the proper wavelength for photosynthesis. Only the reds and blues are useful to plants. Green, ultraviolet and infrared light are not of the proper wavelength (*67*). While 45% of the energy in sunlight is available, only about 0.1 to 3% is actively converted into biomass by the plant (*2*). The rest is used in catabolic respiration processes with release of CO_2, and inherent metabolic inefficiencies. Furthermore, only about one-half of the biomass is available for food or

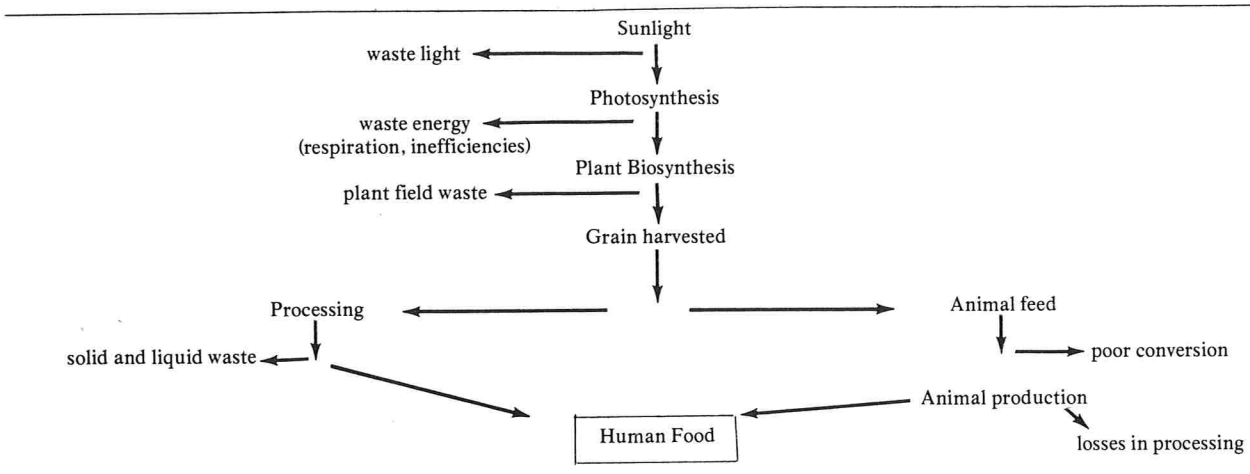
feed. Using corn as an example, only about 0.4% of the energy in sunlight that impinges on the plant's leaves is harvested as food or feed.

About 40% of the food energy in our diet comes from meat and animal products which are produced relatively inefficiently from plants. For example, when feed is expressed in kg of corn per kg of live weight gained, broilers consume 2.4, hogs 5.6, and cattle 11.1 (*2*). Thus, it appears that 40% of our diet is derived from animals that consume 80% of the energy available in harvested crops. While there is serious research to increase yields and the photosynthetic efficiency of plants and the feed conversion efficiency of animals (*21,85*), we may reach certain biological limits which result in wasted calories or energy. Comparing efficiencies throughout the food chain, it is evident that the energy utilization by the plant is by far the most inefficient or wasteful. However the energy wasted, sunlight, does the least damage to the environment. On the other hand, inefficiencies in every other step in the food chain result in *biological* waste. While some biological wastes are apparently unavoidable due to inherent biological inefficiencies (i.e. not all of the corn plant is edible) some wastes can be avoided by application of appropriate processing technologies.

REGULATIONS AND FOOD PROCESSING

The inefficiencies of each step in the food production-consumption process result in waste which may or may not have an environmental impact. For example, if the processing wastes from vegetables are left in the field, the least impact on the environment may be experienced. However, if an inferior product (bruised or diseased) reaches the processing plant, some of the product will contribute to the organic waste load of the waste water (*39*). It is these biological wastes which cause the most serious environmental impact. Accumulation of both liquid and solid wastes in waterways causes severe changes in the microhabitat of the normal communities of plants and animals. Increased organic loads in streams may surpass the streams' normal oxidative capacity and dramatically change population balances (*49*). These imbalances result in undesirable changes in the waterways and are the subject of much concern and the basis for environmental regulation through public laws.

Reduction of the impact of wastes on the environment has been the goal of the Environmental Protection Agency and has been the underlying philosophy of many state and federal laws passed (*72*). The goal of Public Law 92-500 passed in 1972 (*23*) was to restore and maintain the chemical, physical and biological integrity of the native waters. It was to provide for fishable and swimmable waters by 1983 and for zero discharge of pollutants into waterways by 1985 (*22*). To reach these goals a plan was implemented to have industries meet these standards by the best practical technology. Further legislation in 1977 (Clean Water Act of 1977) changed the pathway to achieve the 1985 goals to include an economic factor. Also, the laws now include a provision for waste classification into three control groups: toxic compounds, conventional pollutants and non-conventional pollutants. Emphasis is now placed on the toxic substances discharged (heavy metals, etc.) and is of less concern to food processors (*72*). Most food processors fit the second category by producing conventional pollutants. For these pollutants, biochemical oxygen demand (BOD_5), suspended solids (TSS), pH, and fecal coliforms are regulated. These pollutants must be controlled using readily available reasonable technology that meet the cost test. The cost test is based on two interrelated factors (*a*) the reasonableness of the relation between cost and



TOTAL ENERGY BALANCE[a]

10^{12} calories light/3 mo.	Human food,	4×10^7 calories as corn + 6×10^4 calories wasted
	Animal feed,	2×10^6 calories as corn + 8×10^5 calories wasted

[a]Calories calculated on the bases of production of corn for human consumption directly or as beef when corn is used a ration.

Figure 1. *Energy flow in the food system. Adapted from Agricultural Production Efficiency* (*2*).

the effluent reduction benefits, and (*b*) comparison of the industry category or subcategory cost and associated pollutant reduction for publicly owned treatment plants (*72*).

ALTERNATIVE APPROACHES FOR INCREASED EFFICIENCIES

Change demand

Reduction of waste and subsequent environmental protection through increased efficiencies have been the goals of the food industries, not only because of legislative mandates, but also because of increased economic incentives to do so. Alternatives for waste abatement should be examined for the entire food system, as well as from the food processing viewpoint (Fig. 2). First, it may be possible to change consumer demand so that people consume products that generate less waste in their production. For example, the low efficiency of producing animals for human food from corn has been discussed (*21*). Others have emphasized the desirability of single cell protein as an alternative protein source (*77*). However, most consumers will not change their eating habits to eat less meat as long as they can afford to purchase it.

Change production processes

A second alternative approach for waste abatement is to change the production process (Fig. 2). For example, many changes in the food production system have resulted in increased yields of foods from plants and animals. These include high yielding plant varieties (*15*) which have a higher proportion of edible than of inedible plant parts, thus producing less waste. Higher animal feed conversion ratios also generate less waste (as manure, etc.) and thereby increase the overall efficiencies of food production and economic value for consumers.

Food scientists are normally concerned with what happens after the food is grown and have contributed greatly to increased efficiencies in food processing. Post-harvest technology, the first step in maintenance of food value, has resulted in higher quality farm produce reaching the consumer. Advances have included controlled atmosphere storage for fruits and vegetables (*54*), and locating meat processing plants close to animal production facilities. These practices result in less waste because the product of highest quality reaches the consumer and the maximum yield reaches the processing plants. This is the first place in the total processing scheme for waste reduction, and has received considerable research emphasis. Also, most field waste from fruits and vegetables is now routinely left in the field, resulting in less waste at the processing plants. These practices all combine to give the highest quality product delivered to consumers or to processing plants (*39*) at a reasonable cost.

Change processing

Once the food reaches the processing plant, waste production and treatment becomes a critical issue because large amounts of waste are generated in one place and have locally high impact (*39*). Each industry generates different concentrations and types of waste materials (Table 1). Identification of the amount and composition of the waste generated in different kinds of food processing industries is the first step in a waste utilization or reduction approach and has been the subject of numerous reports (*3,8,9,10,32,37,62,68*). While complete input-output data are not always available, it is possible to generalize waste load characteristics from these data (Table 1). Meat processing results in tremendous quantities of both liquid and solid waste. For example, poultry processing results in solid wastes of 270 g/kg live weight and water usage of 14,000 mg/kg (BOD_5), as well as a very large volume of water used for bird washing and giblet fluming. By contrast, the milk industry produces little solid waste, but very concentrated liquid wastes. Vegetable processing liquid wastes are in general less concentrated, except for starchy wastes such as from potatoes. However, considerable solid waste is generated during normal processing of vegetables.

The most significant approach for waste reduction at the processing plant is to adopt practices and technologies which result in significant improvements

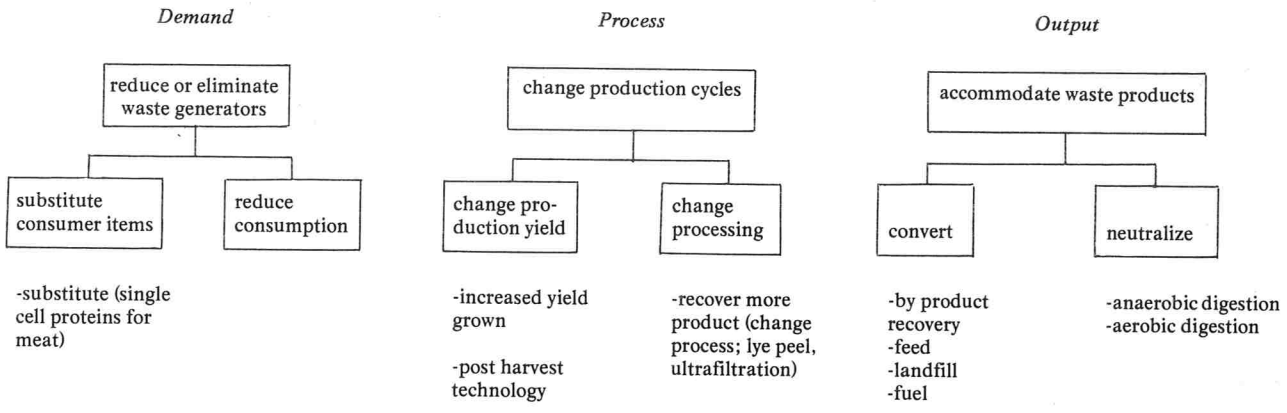


Figure 2. *Waste abatement alternative actions. Adapted from a systems approach to problem-oriented research planning: a case study of food production wastes* (*1*).

in recovery of the final salable product (Fig. 2). While all processors attempt this (to stay in business!), some new applications of technologies have resulted in increased product yield and quality while reducing waste. An example of this approach is the use of ultrafiltered milk to make cheese (Table 2). This process has been demonstrated for several cheese varieties including Mozzarella and Cheddar (*17*), Petit Suisse and Camembert (*50*). The increased yield is due to retention of proteins in the cheese normally excluded in the whey. Since whey is a major pollutant in the dairy industry, this process is an important new application of ultrafiltration technology. The vegetable industry has been successful in adapting new peeling techniques using lye (*80*) and various surfactants (*46*) to peel fruits (*14*) and vegetables (*51*). This results in substantial increases in product recovery (6-40%) not obtainable by mechanical abrasion peeling methods (*83*). Secondly, more varieties of fruits, such as peaches, can be peeled by this method resulting in increased product utilization. This process has received wide recognition and won the 1972 Industrial Achievement Award presented by the Institute of Food Technologists (*54*). It should be noted, however, that lye peeling generates a caustic waste which requires substantial treatment before discharge. The meat industry has increased yields of better quality (more tender) meat to the consumer by enzymatic treatment of animals before slaughter. The ProTen process developed by Swift results in more tender meat cuts and less waste due to inferior meat quality (*54*).

TABLE 2. *Technologies which result in increased yield and decreased waste production.*

Industry	Subclass	Process	Increased yield	Ref.
Meat	Beef	Protein-enzyme tenderization	39%[a]	(*54*)
Dairy	Cheese	Ultrafiltration	16-20%	(*50*)
Fruit and vegetables	Peaches	Lye peeling	Marginal	(*83*)
	Pears	"	Marginal	(*83*)
	Potatoes	"	12-40%	(*51*)
	Pimiento peppers	"	30%	(*9*)
	Apples	"	6%	(*47*)

[a]The increase in yield quoted here is an increase in meat acceptable for dry heat cooking from 29% in a conventional carcass to 68% in a Pro Ten carcass.

Accommodate wastes

While efforts to increase production and processing yields have been made, the most attention has been paid to developing new methods to accommodate waste products (Fig. 2). These have been mostly in two areas: application of processing or recovery technologies to food waste streams, and anaerobic or aerobic waste digestion methods.

For by-product recovery to be successful, the waste water must not vary in composition and must contain a by-product that has some value or special use. Often a nondestructive harvesting technique must be employed for maximum by-product value. Technologies developed for pure chemical separations have been applied to treatment of liquid food processing wastes for by-product recovery. The techniques found useful have included ultrafiltration, reverse osmosis, electrodialysis and ion exchange. For example, processes have been developed for treating potato waste to recover many potentially valuable components from waste water process streams. Researchers at the Eastern Regional Research Center were instrumental in developing ion exchange procedures for recovering amino acids, potassium, organic acids, phosphates and proteins from potato wastewaters. In this process (*35*), the dilute wastes were first concentrated to 2.5% solids by inplant water use modifications, then the concentrate was subjected to ion exchange (*73*) which recovered inorganic cations and precipitated proteins. A second ion exchange recovered proteins and a third recovered organic acids (*35*). The protein recovered (*19*) would be used as an animal feed, and the cations as a fertilizer. A 1971 economic analysis of these processes (*81*), indicated that this scheme was not economically practical at that time. The best alternative was effluent drying of potato process wastes and utilization as animal feed. Protein recovery from

TABLE 1. *Solid and liquid wastes[a] generated during processing of food.*

Processed Food		Total solid waste (g/kg)	Liquid volume (m^3/kg)	BOD_5 (mg/kg)	Reference
Vegetable	Kale	16	0.004	11,000	(*8*)
	Spinach	20		11,000	(*8*)
	Mustard greens	16		10,000	(*8*)
	Turnip greens	15		9,000	(*8*)
	Collards	13		8,000	(*8*)
	Potatoes	66	0.012	44,000	(*10,70*)
	Peppers (lye peel)	65	0.020	33,000	(*9*)
	Tomatoes (lye peel)	14	0.010		(*88*)
Dairy	Cheese whey		9.000	270,000	(*3*)
	Skim milk		0.070	1,500	(*3*)
	Ice cream		0.080	3,000	(*3*)
Meat	Red	0.440	25.000	14,000	(*3*)
	Poultry	0.270	50.000	13,000	(*3*)
	Eggs	0.111			(*32,37*)

[a]Waste loads calculated per unit weight of product.

other processing wastes (e.g. dairy) by ion exchange has also been demonstrated (*65*) but its use is restricted by economic consideration.

Pressure filtration of waste solutions to remove water only (reverse osmosis) or to harvest large molecular weight components such as proteins (ultrafiltration) has also been a technique applied to wastes for by-product recovery. Whey proteins have been successfully harvested by ultrafiltration. In this process, a whey concentrate was produced containing 16% solids, of which 42% was protein (*38*). This protein concentrate could be used as is, or dried for food formulations. Reverse osmosis is usually more difficult to employ because of membrane fouling, but has been developed for recovery of protein from oilseed flour to reduce waste production (*47*) and produce protein isolates. Reverse osmosis has also been used to concentrate citrus centrifuge effluents (*6*). Electrodialysis has been suggested as a useful method for waste treatment. In this process, ionic species can be removed from solution with anion and cation selective membranes and an imposed voltage (*38*). The process has been described for reducing the ionic content of cheese whey proteins.

While these examples of nondestructive harvesting techniques are advantageous because they produce products that have undergone limited destruction, they can be expensive to operate and maintain. Therefore, other less costly harvesting techniques have been often employed. For example, flocculating or precipitating agents can be used to harvest specific large molecular weight products such as proteins from meat packing effluent. The Alwatech process is used commercially to precipitate meat proteins from meat packing effluents using lignosulfonic acid (an approved feed additive) to produce a protein-rich animal feed (*31*). A polymeric flocculating agent, chitosan, has also been suggested as a general flocculant for food processing wastes (*11*). Flocculating agents and precipitants are effective but may have limited uses in food by-product recovery until properly approved as food additives.

Heat or acid and base precipitation of proteins or other components from waste streams can be legally used in human foods, but these treatments can denature or alter the food value of the harvested components. Processes to precipitate proteins from food processing wastes have been demonstrated for meat (*31,36*) and vegetable processing industries (*43,45,52*). In most instances these precipitants are used as animal feeds (*82*).

In some instances the by-product recovered can be used directly in the human food system, e.g. whey proteins (*38*), or be further processed to yield important components such as flavors (*45*). However, in many instances the harvested by-product has little value in human foods and has been used for animal feeds (*41*). This is an excellent use for some human food wastes because food is recycled and maintained in the food system and may act as a substitute for human foodstuffs normally fed to animals. Feeding waste to animals is also desirable because they can often assimilate materials which humans can not. Feeding of both solid and liquid wastes to animals has been widely demonstrated. A silage-like product can be prepared from fruit and vegetable processing wastes (*56*) or solid wastes can be fed directly to ruminants (*34*) or swine (*16*). The liquid wastes from food processing may be harvested, dried and used as poultry or cattle feed. Many reports emphasize the feasibility of this approach to waste abatement (*11,13,31,39,41,45,52,82*). However, as energy costs continue to increase, drying may not be economically feasible.

Growing microorganisms in dilute wastes fortified with suitable nutrients has been suggested as a possible approach to pollution abatement (*7,44*). Often cells are grown until a suitable reduction in dissolved organic matter (BOD_5) is reached. The cells may be harvested by filtration or centrifugation and used as single cell protein (SCP) for animal feed. This process has been suggested by some (*44,78*), and demonstrated for many wastes including whey (*57,58,87*), citric acid waste (*12,40*), lye peeling effluents (*7*), cellulosic wastes (*18,25,29,61,89*), potato waste water (*48,60*), coconut water (*76*), and rice straw (*84*), to name a few. Many of these processes are aerobic and require high energy inputs for aeration. For such costly inputs to be feasible, the conversion to cell mass must be rapid and very efficient and produce a high quality or easily extractible protein. A second valuable by-product of microbial metabolism, such as microbial oil, might also be produced to increase the financial attractiveness of the process (*57,58*). Anaerobic fermentations to produce ethanol are attractive because of the limited aeration necessary to produce a potentially valuable fuel as well as SCP. This process has also been demonstrated for many wastes (*18,25,29,42,63*), but is limited by the cost of distilling the ethanol. An alcoholic fermentation of whey to produce wine has been successfully demonstrated (*66,69*) and would avoid distillation costs.

Conversion of solid wastes to valuable by-products may be preferable to the common practice of landfill treatment (*27*). Silage production is feasible in some cases, as mentioned previously (*56*). Composting of some solid wastes has been suggested for ultimate use as soil conditioners (*27,53*). The potential fuel value of some wastes has been recognized by some (*86,83*), but the practice is limited by the energy expenditure necessary to dry the wastes.

The most common practice in food waste abatement practices has been to try and neutralize the effect of wastes on the environment (Fig. 2). Anaerobic and aerobic digestion and land application facilities have been modeled after traditional sewage treatment practices (*24,71,75,91*). Plants are designed on the basis of waste concentration and volume of flow. Minimization of dissolved organics in waste is important in waste treatment and can result from good plant management practices (*74*). The volume of waste-water produced in

food processing can be substantial. Reductions in volume are obvious for good process control and have been the subject of several national symposia sponsored by the Environmental Protection Agency, and other published reports (*30,33,37,55,68,74,90*).

Treatment of liquid wastes by classical anaerobic and aerobic digestion methods is popular because readily available engineering technologies can be easily applied to waste treatments. However, it is probably the least efficient from a systems point of view, as the waste products are not recycled in the food system as would be the case for use as animal feed or by-product recovery. The relative cost of digestion treatments has nonetheless made this the method of choice. As costs change thru governmental cost incentives (*4*) and increases in value of recoverable by-products, anaerobic and aerobic digestions may become less attractive.

SUMMARY

Waste production in the food system is defined by certain inherent biological limitations such as light conversion efficiencies. The limitations in subsequent processing steps are primarily technological. Many approaches to increase the efficiency of the overall waste utilization process have been developed. The most promising are those that regard waste as a resource and change technologies to harvest the most product (ultrafiltration, etc.). Increased research effort in this area should be emphasized, as it is likely to generate the most economically attractive benefits. When increased product recovery is not possible, the next best alternative is to harvest valuable by-products and recycle these in the food system. Alternatives such as composting, landfill and digestion as sewage may be employed most often but are least desirable from a food utilization and efficiency standpoint.

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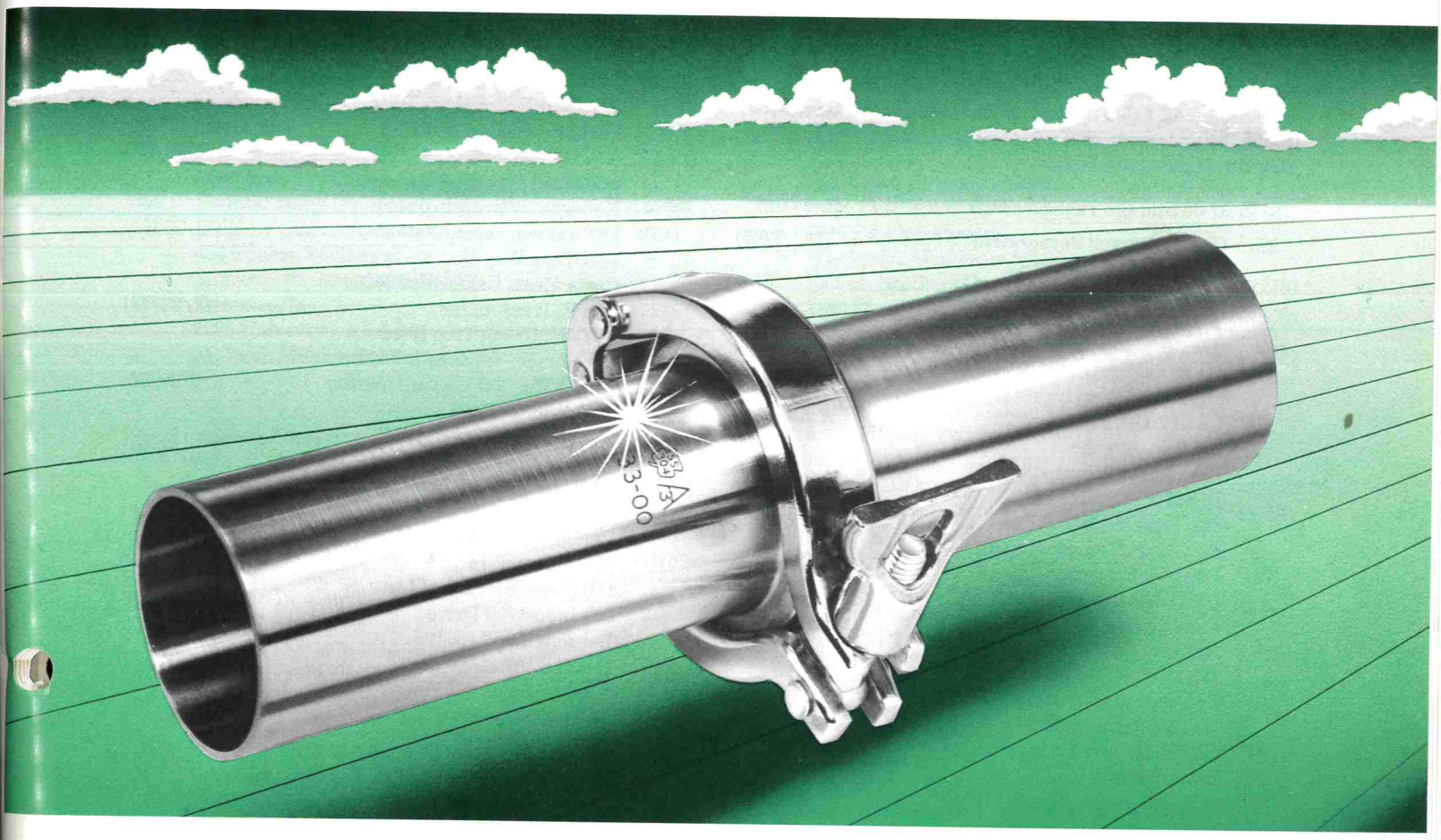
Most consumer advocates were weaned on milk products protected by these symbols.



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S-578

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01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
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575 E. Mill St.
Little Falls, New York 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets
Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C.P. Avenue
Lake Mills, Wisconsin 53551

117 DCI, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

76 Damrow Company (10/31/57)
196 Western Avenue
Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street South
Peterborough, Ontario, Canada
(not available in USA)

109 Girton Manufacturing Company (9/30/58)
State Street
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
P.O. Box 2507
City of Industry, California 91746

127 Paul Mueller Company (6/29/60)
P.O. Box 828
Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-08 Pumps for Milk and Milk Products

325 Albin Motor Aktiebolag (12/19/79)
Box 139, S-681 01 Kristinehamn
Sweden
(not available in USA)

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wisconsin 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd.
Oak Brook, Illinois 60621

29R Cherry-Burrell Corporation (10/ 3/56)
(unit AMCA Int'l)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 CP Avenue
Lake Mills, Wisconsin 53551

205R Dairy Equipment Company (5/22/69)
1919 South Stoughton Road
Madison, Wisconsin 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street
Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way
Costa Mesa, California 92626

314 Len E. Ivarson, Inc. (12/22/78)
3100 W. Green Tree Road
Milwaukee, Wisconsin 53223

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

303 E. R. Mitchell Co., Inc. (3/ 8/78)
(Mfg. by Howard Pneumatic Eng. Co. Ltd.)
576 Haddon Ave.
Collingswood, NJ 08108

319 Mono Group, Inc. (3/21/79)
(Mfg. by SSP Pumps Ltd.)
847 Industrial Drive
Bensonville, IL 60106

241 Puriti S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico
(not available in USA)

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Avenue
Springfield, Ohio 45501

306 Stamp Corp. (5/ 2/78)
1309 Culmen St.
Madison, WI 53713

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 43rd Street
Kenosha, Wisconsin 53140

219 Tri-Canada Ltd. (2/15/71)
P.O. Box 4589
Buffalo, NY 14240

52R Viking Pump Div. (12/31/56)
Houdaille Industries, Inc.
406 State Street
Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
1300 Lincoln Ave.
Waukesha, Wisconsin 53186

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
1241 Rand Rd.
Des Plaines, IL 60016

87 Cherry-Burrell Company (12/20/57)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street
Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
P.O. Box 1441
Minneapolis, Minnesota 55440

309 General Dairy Equipment (7/19/78)
(Mfg. by Rannie A/S, Denmark)
434 Stinson Boulevard
Minneapolis, Minnesota 55413

256 Hercules, Inc. (1/23/74)
2285 University Avenue
St. Paul, Minnesota 55114

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road
Almont, Michigan 48003

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington
Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., Southeast
Minneapolis, Minnesota 55114

66 Dairy Equipment Company (5/29/57)
1919 South Stoughton Road
Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street
Milwaukee, Wisconsin 53235

297 Indiana Tank Co., Inc. (8/29/77)
P.O. Box 366
Simmitt, Indiana 46070

305 Light Industrial Design Co. (3/23/78)
3726 Halverstick Rd.
Sumas, WA 98295

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Avenue
Milton, Ontario, Canada L9T 2Y3
(not available in USA)

85 Polar Tank Trailer, Inc. (12/20/57)
Holdingford, Minnesota 56340

47 Pullman Trailmobile (11/ 2/56)
701 East 16th Avenue
North Kansas City, Missouri 64116

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c.p. 758
Drummondville, Quebec, Canada J2C 2A1
(not available in USA)

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St.
Iberville, Quebec, Canada
(not available in USA)

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-17 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

291 Accurate Metering Systems, Inc. (6/22/77)
1731 Carmen Drive
Elk Grove Village, IL 60007

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue
Waukesha, Wisconsin 53186

245 Babson Brothers Company (2/12/73)
2100 South York Road
Oak Brook, Illinois 60521

284 Bristol Engineering Company (11/18/76)
210 Beaver Street
Yorkville, Illinois 60560

301 Brown Equip. Co., Inc. (12/ 6/77)
9955-9¼ Ave.
Hanford, California 93230

82R Cherry-Burrell Company (12/11/57)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52406

260 CREPACO, Inc. (5/22/74)
100 CP Avenue
Lake Mills, Wisconsin 53551

322 The DeLaval Company Ltd. (7/16/79)
113 Park St. So.
Peterborough, Ontario
Canada K9J 3R8

304 EGMO Ltd-Israel (3/16/78)
(Martin Silver P.E.)
406 Kinderamack Rd.
River Edge, NJ 07661

271 The Foxboro Company (3/ 8/76)
Neponset Street
Foxboro, Massachusetts 02035

67R G & H Products, Inc. (6/10/57)
5718 52nd Street
Kenosha, Wisconsin 53140

203R ITT-Grinnell Company, Inc. (11/ 7/68)
DIA-FLO Div
33 Centerville Rd.
Lancaster, Pennsylvania 17603

34R Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

287 Koltek OY (1/14/77)
Kotinummentieiz
SF-00700 Helsinki 70
Finland
(not available in USA)

239 LUMACO (6/30/72)
Box 688,
Teaneck, New Jersey 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Box 828
Springfield, Missouri 65801

295 Precision Stainless Products (8/11/77)
(Mfg. by Toyo Stainless Co. Ltd.)
5636 Shull St.
Bell Gardens, CA 90201

242 Puriti, S.A. (9/12/72)
Alfredo Nobel #39 Industrial Pte de Vigas
Tlalnepantla, Mexico
(not available in USA)

149R Q Controls (5/18/64)
Occidental, California 95465

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street
Kenosha, Wisconsin 53140

300 Superior Stainless, Inc. (11/22/77)
211 Sugar Creek Rd.
P.O. Box 622
Delvan, Wisconsin 53115

191R Tri-Canada, Ltd. (11/23/66)
P.O. Box 4589
Buffalo, NY 14240

250 Universal Milking Machine (6/11/73)
Div. of Universal Cooperatives
407 First Ave, So.
Albert Lea, Minnesota 56007

278 Valex Products (8/30/76)
20447 Nordhoff St.
Chatsworth, California 91311

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

321 Anderson Instrument Co., Inc. (6/14/79)
R.F.D. #1, Fulton, New York 12072

315 Burns Engineering, Inc. (2/ 5/79)
10201 Bren Road, East
Minnetonka, MN 55343

206 The Foxboro Company (8/11/69)
Neponset Avenue
Foxboro, Massachusetts 02035

285 Tank Mate Company (12/ 7/76)
2269 Ford Parkway
St. Paul, Minnesota 55116

32 Taylor Instrument Process Control (10/ 4/56)
Div. Sybron Corporation
95 Ames Street
Rochester, New York 14601

246 United Electric Controls (3/24/73)
85 School Street
Watertown, Massachusetts 02172

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

296 L. C. Thomsen & Sons, Inc. (8/15/77)
1303 43rd St.
Kenosha, Wisconsin 53140

11-03 Plate-type Heat Exchangers for Milk and Milk Products

316 Agric Machinery Corp. (2/ 7/79)
P.O. Box 6
Madison, NJ 07940

326 American Vicarb Corporation (2/ 4/80)
(Mfg by Vicarb S. A. France)
1522 Main Street
Niagra Falls, N.Y. 14301

20 A.P.V. Company, Inc. (9/ 4/56)
395 Fillmore Avenue
Tonawanda, New York 14150

30 Cherry-Burrell Corporation (10/ 1/56)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets
Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53551

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street
South Peterborough, Ontario, Canada
(not available in USA)

279 The Schluter Co. (8/29/76)
(Mfg. by Samuel Parker Ltd.)
112 E. Centerway
Janesville, WI 53545

17 The DeLaval Separator Company (8/30/56)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

15 Kusel Dairy Equipment Company (8/15/56)
820 West Street
Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264
Bradford, Pennsylvania 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road
Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street
Chester, Pennsylvania 19013

307 G&H Products, Inc. (5/ 2/78)
5718-52nd St.
Kenosha, WI 53141

217 Girton Manufacturing Co. (1/23/71)
Millville, Pennsylvania 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455
Ferndale, California 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828
Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 188
Mora, Minnesota 55051

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
(Mfg. by CREPACO, Inc.)
2100 S. York Road
Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 CP Ave.
Lake Mills, Wisconsin 53551

119R DCI, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

4R Dairy Equipment Company (6/15/56)
1919 South Stoughton Road
Madison, Wisconsin 53716

92R Alfa-Laval Limited (12/27/57)
350 Dutchess Turnpike
Poughkeepsie, N.Y. 12602

49R The DeLaval Separator Company (12/ 5/56)
350 Dutchess Turnpike
Poughkeepsie, N.Y. 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd.
El Monte, California 91732
(not available in USA)

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road
Preston, Ontario, Canada
(not available in USA)

12R Paul Mueller Company (7/31/56)
P.O. Box 828
Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
293 Como Ave.
St. Paul, Minnesota 55103

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

254 Anhydro, Inc. (1/ 7/74)
165 John Dietsch Square
Attleboro Falls, Massachusetts 02763

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street
Buffalo, New York 14207

263 C. E. Howard Corporation (12/21/74)
240 N. Orange Avenue
City of Industry, California 91746

107R C. E. Rogers Company (8/ 1/58)
P.O. Box 118
Mora, Minnesota 55051

277 ConTherm Corp. (8/19/76)
P.O. Box 352
Newbury Port, MA 01950

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road
Birmingham, Michigan 48010

273 Niro Atomizer Inc. (5/20/76)
9165 Rumsey Road
Columbia, Maryland 21044

299 Stork Food Machinery, Inc. (11/16/77)
(Mfg. by Stork-Friesland B.V.)
P.O. Box 816
Somerville, New Jersey 08876

311 Wiegand Evaporators, Inc. (8/28/78)
5585 Sterrett Place
Columbia, Maryland 21044

17-06 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
(unit AMCA Int'l)
2400 Sixth St., Southwest
Cedar Rapids, IA 52404

324 ERCA (11/29/79)
B. P. 54 Z. I. de Courtabeouf
Avenue de Pacifique
91402 Orsay France
(not available in USA)

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge,
Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Div. (4/24/71)
2285 University Ave.
St. Paul, Minnesota 55114

281 Purity Packaging Corporation (11/ 8/76)
4190 Fisher Road
Columbus, Ohio 43228

211 Steel & Cohen (Twin-Pak, Inc.) (2/ 4/70)
745 Fifth Avenue
New York, New York 10022

19-03 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

286 O.G. Hoyer, Inc. (12/ 8/76)
201 Broad St.
Lake Geneva, WI 53147
(Mfg. by O.G. Hoyer A/S of Denmark)

146 Cherry-Burrell Company (12/10/63)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

141 CREPACO, Inc. (4/15/63)
100 CP Avenue
Lake Mills, Wisconsin 53551

22-04 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, New York 13365

154 CREPACO, Inc. (2/10/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

160 DCI, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

181 Damrow Company, Division of DEC (5/18/66)
International, Inc., 196 Western Ave.
Fond du Lac, Wisconsin 54935

262 DeLaval Company Limited (11/11/74)
113 Park Street
South, Peterborough, Ontario, Canada

156 C. E. Howard Corporation (3/ 9/65)
240 N. Orange Ave., Box 2507
City of Industry, CA 91746

155 Paul Mueller Co. (2/10/65)
P.O. Box 828
Springfield, Missouri 65801

312 Sanitary Processing Equip. Corp. (9/15/78)
Butternut Drive
East Syracuse, New York

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-01 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road
Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc., 869 S. Knowles Ave.
New Richmond, Wisconsin 54017

302 Eskimo Pie Corp. (1/27/78)
530 E. Main St.
Richmond, Virginia 23219

258 Hercules, Inc. (2/ 8/74)
2285 University Ave.
St. Paul, Minnesota 55114

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, New York 13365

158 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

187 DCI, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828
Springfield, Missouri 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

275 Bepex Corporation (7/12/76)
150 Todd Road
Santa Rosa, California 95402

162 Cherry-Burrell Corporation (4/ 5/65)
(unit AMCA Int'l)
575 E. Mill St.
Litlle Falls, New York 13365

159 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

188 DCI, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

167 Paul Mueller Co. (4/26/65)
Box 828
Springfield, Missouri 65801

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wisconsin 53950

26-01 Sifters for Dry Milk and Dry Milk Products

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard
Mt. Vernon, New York 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, NY 14211

185 Rotex, Inc. (8/10/66)
(Mfg. by Orville Simpson Co.)
1230 Knowlton St.
Cincinnati, Ohio 45223

176 Koppers Company, Inc. (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6033 E. Bandini Blvd.
Los Angeles, California 90051

27-01 Equipment for Packaging Dry Milk and Dry Milk Products

313 WPM Systems, Inc. (10/10/78)
Div. of St. Regis Paper Company
4990 Acoma St.
Denver, Colorado 80216

28-00 Flow Meters for Milk and Liquid Milk Products

272 Accurate Metering Systems, Inc. (4/ 2/76)
1731 Carmen Drive
Elk Grove Village, Illinois 60007

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road
Milwaukee, Wisconsin 53223

223 C-E IN-VAL-CO, Division of Combustion (11/15/71)
Engineering, Inc.
P.O. Box 556, 3102 Charles Page Blvd.
Tulsa, Oklahoma 74101

265 Electronic Flo-Meters, Inc. (3/10/75)
1621 Jupiter Rd.
Garland, TX 75042

226 Fischer & Porter Company (12/ 9/71)
County Line Road
Warminster, Pennsylvania 18974

224 The Foxboro Company (11/16/71)
Neponset Avenue
Foxboro, Massachusetts 02035

320 Max Machinery, Inc. (3/28/79)
1420 Healdsburg Ave.
Healdsburg, CA 95448

270 Taylor Instrument Company Division (2/ 9/76)
Sybron Corporation, 95 Ames Street
Rochester, New York 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
(Mfg. by CREPACO, Inc.)
2100 S. York Road
Oak Brook, Illinois 60521

31-00 Scraped Surface Heat Exchangers

274 Contherm Corporation (6/25/76)
P.O. Box 352
Newburyport, Massachusetts 01950

322 Cherry Burrell (7/26/79)
2400 6th St. SW
Cedar Rapids, IA 52406

290 CREPACO, Inc. (6/15/77)
100 So. CP Ave.
Lake Mills, WI 53551

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, (1/27/75)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, NY 13365

268 DCI, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

33-00 Polished Metal Tubing for Dairy Products

310 Allegheny Bradford Corporation (7/19/78)
P.O. Box 264
Bradford, PA 16701

289 Ladish Co., Tri-Clover Division (1/21/77)
9201 Wilmot Road
Kenosha, Wisconsin 53140

308 Rath Mfg. Co. Inc. (6/15/77)
2505 Foster Ave.
Janesville, WI 53545

35-00 Continuous Blenders

292 Waukesha Foundry Div. ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

36-00 Colloid Mills

293 Waukesha Foundry Div., ABES Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

37-00 Pressure and Level Sensing Devices

318 Anderson Instrument Co., Inc. (4/ 9/79)
R.D.#1 Fulton, N.Y. 12072

317 C-E Invalco Division of Combustion (2/26/79)
Engineering, Inc.
P.O. Box 556
Tulsa, OK 74101

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interested in and they should include appropriate information on their current affiliation and qualifications.

Participation in the consensus procedure

Before acceptance of the contents of the 15th Edition of the *Standard Methods for the Examination of Dairy Products* there will be an opportunity for interested individuals to provide reactions to the text. Participants will be asked to monitor changes as published in the *Journal of Food Protection* or as received from the APHA office. Responses will be obtained through returning evaluation forms sent to volunteers.

The Technical Committee will receive all consensus comments and make appropriate improvements. All comments associated with nonconsensus must be accompanied by written reasons. The Committee reserves the right to incorporate changes or make no changes as the facts warrant. Participants will be contacted regarding suggestions as appropriate.

This procedure has been used in the preparation of other American Public Health Association publications and has helped produce better methods and writeups and to minimize misunderstandings for users.

Those desiring to participate in either chapter committee work or in the consensus procedure should communicate their desires to either of the following:

G. H. Richardson
UMC 87
Utah State University
Logan, UT 84322

Mrs. Seiko Brodbeck
Assoc. Executive Director
APHA
1015 Fifteenth St., N.W.
Washington, DC 20036

Proposals Requested to Study Methods for Analysis of Milk and Milk Products

The Technical Committee on *Standard Methods for the Examination of Dairy Products*, in an attempt to improve analytical procedures, has identified some areas that need further study. The Committee has some funds available to support such studies and is inviting interested investigators to submit proposals for the work.

Proposals should be short (5-7 double-spaced typewritten pages) and should include the following: (*a*) brief review of pertinent literature, (*b*) objectives of the study, (*c*) procedures to be used in the study, (*d*) budget, (*e*) time required for the study and when results can be expected and (*f*) list of references cited in the proposal. Funds are limited and hence the budget should be largely restricted to supplies. In some instances funds for labor may be available.

ELEVEN COPIES OF COMPLETED PROPOSALS SHOULD BE SENT BY JULY 15, 1980 TO: DR. H. L. BODILY, P.O. BOX 69, MIDWAY, UTAH 84049. Questions about technical matters related to a given proposal should be addressed to the person whose name, address, and telephone number appear with each of the areas to be investigated. Areas of investigation

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Coming Events/News and Events

March 17-19--FOOD MICROBIOLOGY. Three-day course, sponsored by Center for Professional Advancement. Central New Jersey location for course. Registration fee: $490. Contact: Mary Sobin, Information Services, PO Box H, Center for Professional Advancement, PO Box 964, East Brunswick, NJ 08816.

March 17-19--CANADIAN FOOD PLANT SANITATION SEMINAR. Toronto, Ont. Sponsored by American Institute of Baking and Bakery Council of Canada. Contact - Carol Lyon, AIB, 1213 Bakers Way, Manhattan, KS 66502, 913-537-4750.

March 19--INDIANA DAIRY INDUSTRY CONFERENCE. Purdue University. Joint Annual Meeting of the Dairy Technology Societies of Indiana will be at The Trails following Conference. Contact: James V. Chambers, Dairy Technology Extension Specialist, Animal Sciences Dept., Purdue University, West Lafayette, IN 47907.

March 24--IOWA ASSOCIATION OF MILK AND FOOD SANITARIANS, ANNUAL MEETING. Gateway Center Motel, Ames. Contact: Bill LaGrange, Dept. of Food Technology, Iowa State University, Ames, IA 50011.

March 24-26--FOOD MICROBIOLOGY. shortcourse. Sponsored by the Center for Professional Advancement. Contact - Mary Sobin, Dept. NR, Center for Professional Advancement, PO Box H, East Brunswick, NJ 08816, 201-249-1400.

March 24-28--MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. The Ohio State University, Columbus, OH. Contact - J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., The Ohio State University, Columbus, OH 43210.

March 25--DAIRY INDUSTRY CONFERENCE. Scheman Building, Iowa State University, Ames, IA. Contact - V. H. Nielsen, Dept. of Food Technology, Iowa State University, Ames, IA 50011.

March 25-26--WESTERN FOOD INDUSTRY CONFERENCE. Freeborn Hall, University of California, Davis. Contact: John C. Bruhn, Dept. of Food Science & Technology University of California, Davis, CA 95616, 916-752-2192.

March 25-27--FLORIDA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, ANNUAL MEETING, in conjunction with University of Florida. Student Union, Univ. of Florida, Gainesville, FL. Contact: W. A. Brown, 508 Mayo Bldg., Tallahassee, FL 32304, 904-487-1450.

March 26--ONTARIO FOOD PROTECTION ASSOCIATION, ANNUAL MEETING. Holiday Inn, 970 Dixon Road, Toronto, Ont. Contact: Gail Holland, Meat Packers Council of Canada, 5233 Dundas St. W., Islington, Ont. M9B 1A6.

Mar. 26-28---CONFERENCE ON WASTEWATER TREATMENT TECHNOLOGIES FOR THE CONTROL OF TOXIC/HAZARDOUS POLLUTANTS. Stouffer's Cincinnati Towers, Cincinnati, OH. Contact: Kenneth A. Dostal, IERL-Ci, EPA, Cincinnati, OH 45268.

March 26-28--GOOD MANUFACTURING PRACTICES (GMP) FOR THE FOOD INDUSTRY. Three-day course, sponsored by Center for Professional Advancement. Central New Jersey location for course. Registration fee: $490. For further information, see March 17-19 entry.

Mar. 27-28---1980 MEAT INDUSTRY RESEARCH CONFERENCE. Ramada O'Hare Inn, Chicago, IL. Contact: Dr. John Birdsall, Director of Scientific Activities, American Meat Institute, P.O. 3556, Washington, DC 20007.

March 31-April 2--1980 AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE'S "KULTURES AND KURDS KLINIC." Hilton Airport Plaza Inn, Kansas City, MO. National judging contest will be held in conjunction with Klinic. Contact: Dr. C. Bronson Lane, ACDPI, PO Box 7813, Orlando, FL 32854 or Margie Franck, ACDPI, 910 17th St., NW, Washington, DC 20006.

April 7-10--MISSOURI MILK, FOOD AND ENVIRONMENTAL HEALTH ASSOCIATION, ANNUAL MEETING. Ramada Inn, Columbia, MO. Contact: Erwin Gadd, Missouri Division of Health, PO Box 570, Jefferson City, MO 65102, 314-751-2335.

April 13-16--SECOND ANNUAL CONFERENCE ON INDUSTRIAL ENERGY CONSERVATION TECHNOLOGY. Hyatt Regency Hotel, Houston, TX. Co-sponsored by Texas Industrial Commission and the U.S. Dept. of Energy. Contact: M. A. Williams, Technical Program Director, or Gerald Brown, Executive Director, Texas Industrial Commission, Box 12728, Austin, TX 78711, 512-472-5059.

April 14-16---5th ANNUAL FOOD SERVICE SYSTEMS SEMINAR AND EXPOSITION. Sheraton O'Hare Hotel, Chicago, IL. Contact: G. E. Livingston, Food Science Associates, Inc., 595 Fifth Avenue, New York, NY.

Comments on Compendium Requested

The APHA is currently planning for preparation of a second edition of the *Compendium of Methods for the Microbiological Examination of Foods.* It is expected that there will be about 57 chapters covering subjects similar to those in the first edition. The APHA invites comments on changes for improvement of the volume. The APHA also desires to know of persons who may be available for assistance with the preparation or review of manuscripts.

Comments or indications of availability regarding specific chapters or areas should be forwarded to Howard L. Bodily, Ph.D., P.O. Box 69, Midway, Utah, 84049 as promptly as possible but no later than June 15, 1980.

Announcement and Invitation to Participate: SMEDP-15

The Technical Committee of the American Public Health Association responsible for developing *Standard Methods for the Examination of Dairy Products* is inviting interested individuals to participate in preparation of the 15th Edition. Participation can be in two areas, chapter preparation and review, and arrival at consensus.

Participation in chapter preparation

The Technical Committee will soon be considering chapter committee composition and welcomes participation from those who have important suggestions and contributions relative to methods that should be included, excluded or modified in the 15th Edition. Volunteers should indicate the specific chapter(s) (as found in the 14th edition) they are

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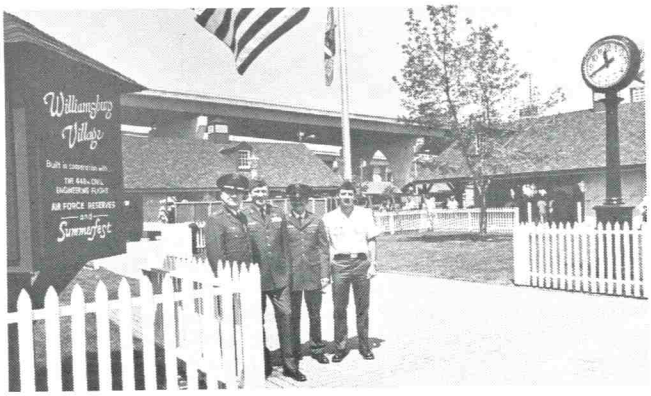
Positions Available

The University of Vermont invites applications for the position of Chairman of the Department of Animal Sciences to begin on or before Sept. 1, 1980. Candidates should have a Ph.D. Degree in Dairy, Poultry, Animal Science, or related field; excellent academic credentials; the highest standards of productivity; and a significant record of accomplishments. The candidate should be able to relate to the practical problems and opportunities of the Vermont animal industry and to the proper role of the Department, College, University in the traditional land-grant mission. Salary will be competitive and commensurate with qualifications. Send complete resume and names of three references by June 1, 1980 to Dr. H. W. Vogelmann, Chairman, Search Committee, Marsh Life Science Building, University of Vermont, Burlington, Vermont 05405. An Equal Opportunity Affirmative Action Employer.

Health Officer, City of Moline, IL. Administrative and technical position in the City enforcing city and state health rules and regulations. Has direct supervision over inspection program of all food and drink handling establishments, day care centers, enforcement of Grade "A" Milk Program, and all activities of the animal control shelter. Oversees handling of all complaints pertaining to weed control, rat and rodent infestations, animal and other nuisances. Must have graduated from a college or university with major work in physical sciences, preferably bacteriology or chemistry, and be able to supervise a milk and water laboratory; have experience as a sanitarian; and hold a Registered Sanitarian license for the State of Illinois. Salary range, $21,696-$24,888. Added benefits. Applicants should apply to the City Administrator's Office, City Hall, 619-16th St., Moline, IL 61265. An Equal Opportunity Employer.

Index to Advertisers

An Invitation from America's Dairyland:



For professionalism and Gemütlichkeit, plan to attend the 67th Annual Meeting of IAMFES, July 27-31, 1980 at the Red Carpet Hotel, Milwaukee, Wisconsin.

Gemütlichkeit Abendgesellschaft
Monday, July 28, 1980, 6-10 p.m.
Abendgesellschaft = Evening Party
Gemütlichkeit = Well, it's a word not easily defined. Some say it's the feeling Milwaukeeans get when plates are heaped with food, beer is flowing freely, and someone from out of town is there to pick up the check. Come and enjoy, then offer your own definition of Gemütlichkeit!
The IAMFES 1980 meeting will be held in conjunction with NEHA's Annual Educational Conference. Reciprocal admission between IAMFES scientific sessions and NEHA educational sessions has been authorized by the executive boards of both groups.
Don't miss this opportunity to participate in the 67th Annual Meeting of IAMFES!

1980 IAMFES ANNUAL MEETING

Advance Registration Form for the 67th Annual Meeting, July 27-31, 1980, Milwaukee, Wisconsin

MAIL TO: **Richard Rowley, Chairman of Registration**
IAMFES
Bureau of Consumer Protection and Environmental Health
Milwaukee Health Department
P.O. Box 92156
Milwaukee, Wisconsin 53202

Please check where applicable:
☐ Affiliate Delegate ☐ Speaker ☐
☐ Past President ☐ Host ☐
☐ Executive Board ☐ Non-member ☐

Make checks payable to:
IAMFES 1980 Meeting Fund

Advance register and save - refundable (prior to June 31) if you don't attend

ADVANCE REGISTRATION FEE (prior to July 1)
(*All in American currency*)

	*Member**	*Spouse of Member*	*Student*
Registration	$20.00	$ 8.00	No chg.
Banquet	15.00	15.00	$15.00
Gemütlichkeit Abendgesellschaft	2.00	2.00	2.00
Total	$37.00	$25.00	$17.00

REGISTRATION FEE AT DOOR
(*All in American currency*)

Member	*Spouse of Member*	*Student*	*Nonmember*
$25.00	$11.00	No chg.	$30.00
17.50	17.50	$17.50	22.50
3.00	3.00	3.00	5.00
$45.50	$31.50	$20.50	$57.00

**Member IAMFES or Wisconsin Dairy Plant Fieldmen's Association*

Name (Member) _____ Spouse _____
Children's First Names and Ages _____
Employer _____
Address _____
City _____ State _____ Zip _____
Means of Transportation _____

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Milwaukee, Wisconsin 53207
Telephone 414-481-8000

Reservations must be received by June 26, 1980
Deposit of 1 night's lodging plus 9% tax or a major credit card number required.
Check in after 1:00 P.M.
Check out time is 12:00 Noon

Arrival Date _____ Departure Date _____
Arrival Time _____ Means of Transportation _____
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City _____ State _____ Zip _____

Please check type of accommodation required
_____ Single (one person) $38.00
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Family Plan: No charge for children (under 18) when occupying the same room as parents.
Roll-a-way beds available at $4.00 each.

One bedroom and two bedroom suites available.
Courtesy Van operates between Mitchell Field Airport and the Red Carpet Hotel.

Mail directly to: **Red Carpet Hotel,** Reservations
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Milwaukee, WI 53207

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TO DAIRY & FOOD
PROCESSING EQUIPMENT

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U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

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ODORLESS—TASTELESS

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HAYNES-SPRAY
should be used to lubricate:

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HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
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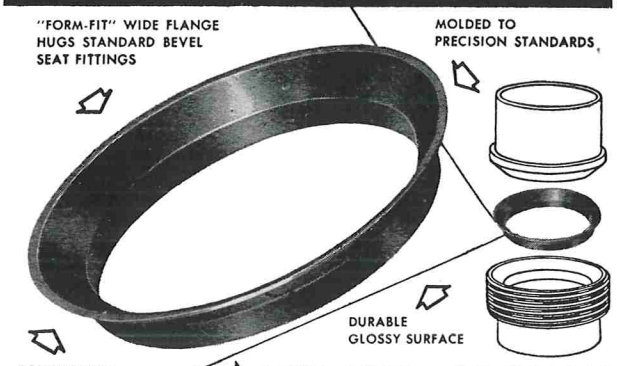
HAYNES SNAP-TITE GASKETS



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- Help overcome line vibrations
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SELF-CENTERING
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SIZES 1" - 1½"
2" - 2½" - 3" - 4"

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THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

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HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

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ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

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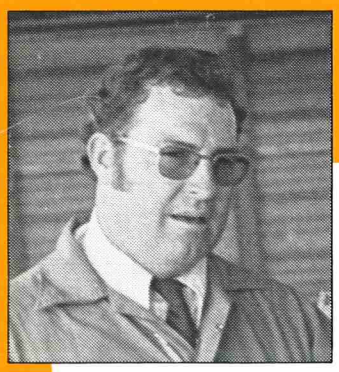
SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY



John E. McCormack, D.V.M., Extension Veterinarian

Dr. McCormack operated a veterinary practice for 10 years in southwest Alabama. He has served on the teaching staff of veterinary colleges at Auburn University, Louisiana State University and the University of Georgia. He has been Extension veterinarian at the University of Georgia since 1976, and has written numerous cattle health articles for dairy publications and conducted many meetings on animal health matters for dairymen.

"The Extension specialist is an educator, a communicator, and a motivator to the dairy industry. Our job is supporting the basic unit of the Cooperative Extension Service; the county agents and their programs. In addition, we help local veterinarians by consulting, reinforcing their educational efforts, and providing updated information on research findings that help them provide better animal health care. Thus, the ultimate goal is to help dairymen, and other livestock owners, reap greater financial rewards from improved health care practices.

"Our educational efforts emphasize positively approached animal health programs.

How We Get Our Message Across

"Our basic educational tool is the dairy association meeting. We attend and present educational material at many of these each year, on a pre-planned basis with the county agent or dairy association official. Sometimes, however, it may be a quickly organized effort to present up-to-date material on an emerging health problem or disease.

"Since we feel that a regular scheduled program of reproductive tract examinations is important for maximum, long-range milk production, we emphasize this facet of dairy herd management. Usually these reproduction seminars or short courses for dairymen are held in the evening, sometimes in conjunction with a planned meal. We make certain the local veterinarians are involved in these programs. Specimens of reproductive organs, both normal and abnormal, are collected from a local slaughterhouse and are layed out on a table for the participants to look over and examine. We point out the normal anatomical structures of the reproductive tract, such as the ovary, and cervix and its rings, and other pertinent structures. Usually, we have several uteri in various stages of pregnancy.

"We encourage the dairymen to palpate these, then we incise the pregnant horn and examine the fetus after everyone has given the estimate of its age. This practical demonstration is supplemented with a slide presentation showing the basics of a reproduction program.

"This type seminar almost always creates a desire among some dairymen to enter into a regular program of reproductive exams. This is why the local veterinarian is an important part of the seminar. We motivate, the practitioner participates and together we instigate the program.

"Other programs in which we are routinely involved are vaccination schedules, parasite control programs and mastitis control. We cooperate with other dairy specialists and quality milk control officials in putting on milker schools and mastitis prevention programs.

"For instance, if high leucocyte counts and mastitis are a problem in an area, we may put on a program emphasizing milking hygiene, basic milking machine function, teat dipping, dry cow therapy and so forth. We ask the dairymen to bring milk to the meeting from a 'clean cow' and a known infected cow and we show them how to run a CMT on that milk.

"Educational meetings are a valuable teaching tool. However, one on one individual farm visits are even more beneficial, but are obviously done on a smaller scale. An example might be a situation where a dairyman has excessive numbers of cows off feed after calving, displaced abomasums, and breeding difficulties. We see this quite often in the form of the fat cow syndrome due to excessive feeding of grain and/or corn silage during the dry period. We cooperate with the local veterinarian on these visits and usually go with him on these trips.

"Telephone consultations are carried on daily with dairymen regarding some health problem or important decision. Examples include interpretation of laboratory information, the wisdom of instituting a BVD vaccination program or how to eliminate a foot disease problem.

"The message of the Extension veterinarian is: by utilizing the knowledge available to him, the dairyman can have healthier, more productive cows. This message is delivered in meetings, written articles, farm visits, and response to telephone calls. We feel a knowledgeable dairyman will be a better dairyman and continue to learn even more about his profession."